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Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*

VI. THE MUTAGENIC CELL STAGE RESPONSE OF THE MALE GERM LINE TO THE 'NITROGEN-MUSTARD' DERIVATIVES OF AMINO-ACIDS, CARBOXYLIC ACIDS AND AMINES

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I. INTRODUCTION

The biochemical mechanism of genetic change is perhaps the most important aspect in the field of mutagenesis, and unfortunately it is the least understood. Chemical mutagens offer an advantage over radiation in the solution of this problem. Mutations induced chemically must be initiated by a reaction: either within the hereditary nucleoproteins, or with a vital molecule in their environment which is involved in gene synthesis or replication. It would seem feasible, therefore, that a comparative analysis of the mutagenic and biochemical properties of closely related compounds would ultimately lead to the solution of the molecular mechanism of mutation.

The quantitative interpretation of the chemical mutagenesis results, however, is charged with difficulties and pitfalls. Apart from the experimental artifacts of administration, there are the biological factors: mainly toxicity and cellular penetration, and also the biochemical complications: due to reactions in the cytoplasm before the active mutagen reaches the genetic material inside the nucleus. In *Drosophila*, the feeding technique is obviously of very limited value in the analysis of the biochemistry of mutagenesis. Reactions are bound to occur between the mutagen and the food constitutents, as well as with the contents of the alimentary canal and haemocoel, before any active compound reaches the gonad, which is the organ that is subjected to the genetic study. In order to exclude some of these unanalysable reactions, we developed our micro-injection technique, whereby the active mutagen is introduced in measured amounts directly round the male germ-cells. The genetic experiments are then designed for the analysis of the influence of the intracellular environment, during sperm differentiation, on the mutagenic yield. This information, coupled with a knowledge of the chemical properties and metabolic fate of the compounds tested, does enable the assessment of the major biological and biochemical factors affecting mutagenicity. The value of this approach has already been demonstrated in our study of the genetic effects of the alkyl methanesulphonates (Fahmy & Fahmy, 1956, 1957*a*). In this communication it is intended to analyse the mutagenic properties of a series of closely related

nitrogen-mustards, with a view to the elucidation of how far slight changes in the chemical structure of a mutagenic molecule could affect the relative mutation yield of the cell-stages of the male germ line.

II. MATERIAL AND TECHNIQUE

The Oregon-K wild type strain of *Drosophila melanogaster* was used for the mutagenic tests. Males were subjected to treatment at an age of 30 ± 5 hours after eclosion, and were of roughly the same size and weight. About 200–500 males were selected for each experiment, and they were then partially dewinged and weighed on an analytical chemical balance. In the present set of experiments the average weight per male was $0{\cdot}85 \pm 0{\cdot}05$ mg. An aqueous solution of the compound under test was prepared, and injected by a calibrated micrometer-syringe around and within the testes of the weighed males, so that each fly received the same volume of solution. The injected males were then reweighed. The difference in the average weight per male before and after injection gave the weight injected, which for dilute solutions is roughly equal to the volume. In the present experiments the average volume of solution received per male was $0{\cdot}3\,\mu$l.

The compounds analysed for mutagenicity in the present study are the 'N-mustard' derivatives of amino-acids and carboxylic acids as well as an aromatic amine. All compounds were synthesized in the Chemistry Department of the Chester Beatty Research Institute; their code numbers, formulae, and full chemical names are given below.

Phenylamino-acid mustards:

CB. 3007, CB. 3025, CB. 3026: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH(NH_2)COOH$;
p-NN-di-(2-chloroethyl)aminophenylalanine, the DL-mixture (Merphalan), the L-isomer (Melphalan), and the D-isomer (Medphalan).

CB. 3051: $(ClCH_2CH_2)_2N.C_6H_4\text{-}O\text{-}C_6H_4.CH_2CH(NH_2)COOH$;
p-/*p*′-NN-di-(2-chloroethyl)aminophenoxy/phenylalanine.

CB. 1385: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH_2CH(NH_2)COOH$;
α-amino-γ-*p*-(NN-di-2-chloroethyl amino) phenylbutyric acid.

Phenylcarboxylic acid mustards:

$(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_nCOOH$

CB. 1331: $n = 1$; *p*-NN-di-(2-chloroethyl)aminophenylacetic acid.

CB. 1332: $n = 2$; *p*-NN-di-(2-chloroethyl)aminophenylpropionic acid.

CB. 1348: $n = 3$; *p*-NN-di-(2-chloroethyl)aminophenylbutyric acid, or 'Chlorambucil'.

CB. 1356: $n = 4$; *p*-NN-di-(2-chloroethyl)aminophenylvaleric acid.

Phenylethylamine mustard:

CB. 3034: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH_2(NH_2)$;
p-NN-di-(2-chloroethyl)aminophenylethylamine.

These compounds are insoluble in water and could, therefore, be administered by injection only as a suspension. Previous experience, however, has shown the gross inadequacy of such a procedure, since it is impossible to ensure the homogeneity of dose per male in the injected volume. The ethylamine-mustard (CB. 3034) was injected in solution as the hydrochloride. All the other compounds were converted to the sodium salts, and were in complete solution at the concentrations utilized in the present experiments. It should be noted, however, that the sodium salts are very unstable, thus necessitating the preparation of the sample for each set of experiments separately, just before administration. Some differences did occur in the activity of the various samples, resulting in some variation in replicate experiments both as regards toxicity and mutagenic response. These variations, however, did not obscure the major mutagenic characteristics of any of the compounds examined.

The mutations analysed in this communication are the sex-linked recessive lethals as detected by the Muller-5 technique. The scoring method used in our laboratory has already been described in detail (Fahmy and Fahmy, 1955). It should be re-emphasized, however, that mutants scored as lethals are those that exert *complete* killing in the hemizygous state. F_2 lethal cultures, therefore, contain males of the Muller-5 genotype ($sc^{81}B\,(InS)\,w^a\,sc^8/Y$) and none of the potential wild type class. Sex-linked recessive visibles were observed in all experiments and will be considered in a later publication.

The progeny of the treated males was fractionated into broods, to enable the assessment of the effect of cell stage on mutagenicity. This has been undertaken by our standard method which has already been described (Fahmy & Fahmy, 1954, 1955). Treated males are mated to a succession of virgin females at 3-day intervals, and the mutation rates in the successive broods are determined separately. Under our experimental conditions, the post-meiotic stages (sperm and spermatids) are predominantly utilized within the first four broods (0–12 days after treatment), whereas the meiotic and premeiotic stages (spermatocytes and spermatogonia) are mainly utilized in later broods (see section IV). In some instances, experimental convenience necessitated slight deviations from the standard progeny fractionation procedure. Such deviations are indicated in the relevant tables in the experimental section.

III. OBSERVATIONS

1. *The amino-acid mustards*

Of the amino-acid mustards studied, the phenylalanine derivatives (the L-isomer, CB. 3025, the D-isomer, CB. 3026, and the DL-mixture, CB. 3007) proved to be the most suitable for the genetic analysis and were used extensively (Tables 1–3 and Fig. 1). The naturally occurring L-isomer gave the most consistent brood-mutation curves (CB. 3025, Fig. 1A). Here it is abundantly clear that the mutagenic response is higher in the later broods. The cytotoxic effect of the compound (as manifested by sterility through lack of sperm) also increases the later is the brood, and only small progenies were recovered from the fourth brood onwards. Sterility through

cytotoxicity is most pronounced with the D-isomer, so that males treated even with low doses become gradually more infertile with time after treatment, and completely failed to produce offspring after the fourth brood. The brood-mutation analysis for the D-isomer, therefore, could only be undertaken for the first 12 days after treatment (Fig. 1B). Though the picture here is not as complete as with the L-isomer, the overall trend seems to be the same: there is a tendency for a higher mutagenic yield

Table 1. *Sex-linked recessive lethals induced by the mustard derivative of* L-*phenylalanine*

Conc. ($\times 10^{-2}$M)	Brood No.*	Chromosomes	Lethals No.	Lethals %
0·8	I	588	17	2·9
	II	426	24	5·6
	III	539	44	8·2
	IV	210	20	9·5
	Total	1763	105	6·0
0·9 (*a*)	I	564	42	7·4
	II	568	53	9·3
	III	306	33	10·8
	Total	1438	128	8·9
(*b*)	I	355	14	3·9
	II	323	16	5·0
	III	250	24	9·6
	Total	928	54	5·8
(*c*)	I	328	22	6·7
	II	185	16	8·6
	III	241	20	8·3
	Total	754	58	7·7
(*d*)	I	360	19	5·3
	II + III	347	41	11·8
	Total	707	60	8·5
(*e*)	I	342	19	5·6
	II + III	350	19	5·4
	Total	692	38	5·5
(*f*)	I	345	20	5·8
	II + III	334	23	6·9
	Total	679	43	6·3
(*g*)	I	515	26	5·0
1·07 (*a*)	I	451	15	3·3
	II	491	29	5·9
	III	161	21	13·0
	IV	71	12	16·9
	V	3	—	—
	Total	1177	77	6·5
(*b*)	I	396	33	8·3
	II	372	37	9·9
	III	179	23	12·8
	IV	45	5	(11·1)
	Total	992	98	9·9
(*c*)	I	74	6	8·1
	II	80	2	2·5
	III	13	1	(7·7)
	Total	167	9	5·4
(*d*)	I	235	33	14·0
1·2	I	531	29	5·5
	II	504	37	7·3
	III	313	24	7·7
	IV	147	11	7·5
	V	85	10	11·8
	VI	11	1	(9·1)
	Total	1591	112	7·0
1·5	I	255	15	5·9
	II	90	14	15·6
	Total	345	29	8·4

*Broods I–VI start, respectively, on days 0, 4, 7, 10, 13, 16.

the later is the brood. With the DL-mixture, the sterilizing effect on the treated males (particularly at low doses) was not very marked, and some offspring was obtained as late as the sixth and seventh broods (16–21 days after treatment). It is also to be noted that the brood-mutation curves for the DL-mixture (Fig. 1C) are more confused than for either of the pure isomers. On the whole, however, the general trend is not incompatible with the picture conveyed by the pure isomers. There is an appreciable mutation rate in all fractions of the progeny, including the late broods. In some experiments with the lower doses, however, the mutation

yield in the later progeny fractions sometimes falls below that occurring in the first. This is partly a sampling error, due to the low rates, and the few viable flies available for the genetic tests in the later broods. In part also, this is the outcome of germinal selection that operates against the recovery of mutants induced in the young gonia. Obviously the role of this selection becomes more apparent at the lower doses, where only a few mutations occur, than at high doses where the high mutation yield due to cell sensitivity, supersedes and conceals the reduction in mutation rate due to

Table 2. *Sex-linked recessive lethals induced by the mustard derivative of* D*-phenylalanine*

Concentration ($\times 10^{-2}$ M)		Broods No.	Broods Days	Chromosomes	Lethals No.	Lethals %
0·8		I	0–3	559	19	3·4
		II	4–6	432	18	4·2
		III	7–9	493	25	5·1
		IV	10–12	115	11	9·6
		Total		1599	73	4·6
0·9		I	0–3	202	14	6·9
		II	4–6	197	18	9·1
		Total		399	32	8·0
1·07	(*a*)	I	0–3	482	31	6·4
		II	4–6	490	44	9·0
		III	7–9	51	3	5·9
		Total		1023	78	7·6
	(*b*)	I	0–3	461	34	7·4
		II	4–6	427	45	10·5
		III	7–9	39	4	(10·3)
		Total		927	83	9·0
1·2		I	0–3	110	16	14·5
		II	4–6	40	3	(7·5)
		III	7–9	29	5	(17·9)
		Total		179	24	13·4
1·5		I	0–3	78	7	9·0
		II	4–6	120	14	11·7
		Total		198	21	10·6

germinal selection. One of the experiments with the DL-mixture (expt. at $0{\cdot}9 \times 10^{-2}$ M, Table 3) was markedly anomalous, yielding the highest mutation rate in the first brood. This is obviously an artifact, probably due to exceptional physico-chemical circumstances leading to excessive penetration of the drug into the sperm. It is of interest to note, however, that even in this anomalous experiment, the mutation yield in the late progeny (recovered in the fifth and sixth broods) was reasonably high, confirming the positive response of the young germ-cells.

The statistical analysis of the fluctuation in mutation yield in successive broods is presented in Table 4. The relative frequency of mutant to normal chromosomes for

Table 3. *Sex-linked recessive lethals induced by the mustard derivative of* DL-*phenylalanine*

Conc. ($\times 10^{-2}$M)	Brood No.*	Chromosomes	Lethal No.	Lethal %
0·45	I	406	13	3·2
	II	441	11	2·5
	III	433	20	4·6
	IV	456	15	3·3
	V	204	4	2·0
	VI	137	1	0·7
	VII	34	—	—
	Total	2111	64	3·0
0·51	I	458	7	1·5
	II	457	12	2·6
	III	434	6	1·4
	IV	444	10	2·3
	Total	1793	35	2·0
0·8	I	466	11	2·4
	II	465	7	1·5
	III	216	2	0·9
	Total	1147	20	1·7
0·9	I	377	43	11·4
	II	448	18	4·0
	III	459	18	3·9
	IV	361	24	6·6
	V	217	9	4·1
	VI	54	4	7·4
	Total	1916	116	6·1
1·2	(*a*) I	555	12	2·2
	II	563	35	6·2
	III	282	29	10·3
	IV	244	20	8·2
	V	167	13	7·8
	VI	34	1	(2·9)
	VII	6	2	—
	Total	1851	112	6·1
	(*b*) I	342	18	5·3
	II	245	19	7·8
	III	69	9	13·0
	IV	4	—	—
	Total	660	46	7·0
1·5	(*a*) I	176	22	12·5
	II	125	15	12·0
	III	21	3	(14·3)
	Total	322	40	12·4
	(*b*) I	434	41	9·4
	II	451	33	7·3
	Total	885	74	8·4

*Broods I–VII start, respectively, on days 0, 4, 7, 10, 13, 16, 19.

Table 4. *Distribution of χ for the increase in mutation rate between consecutive broods under the mustard derivative of phenylalanine*

	Broods I–II	II–III	III–IV	IV–V	V–VI
L-isomer					
Total χ	9·9437	9·3133	0·9885	1·0954	−0·0265
No. of estimations	9	8	4	1	1
P	0·0005	0·0005	0·31	0·14	0·48
D-isomer					
Total χ	4·0410	1·0985	1·8385		
No. of estimations	6	4	1		
P	0·05	0·29	0·03		
DL-mixture					
Total χ	−1·1131	3·4498	0·8800	−2·3472	−0·9323
No. of estimations	8	7	4	3	3
P	0·35	0·10	0·33	0·09	0·29

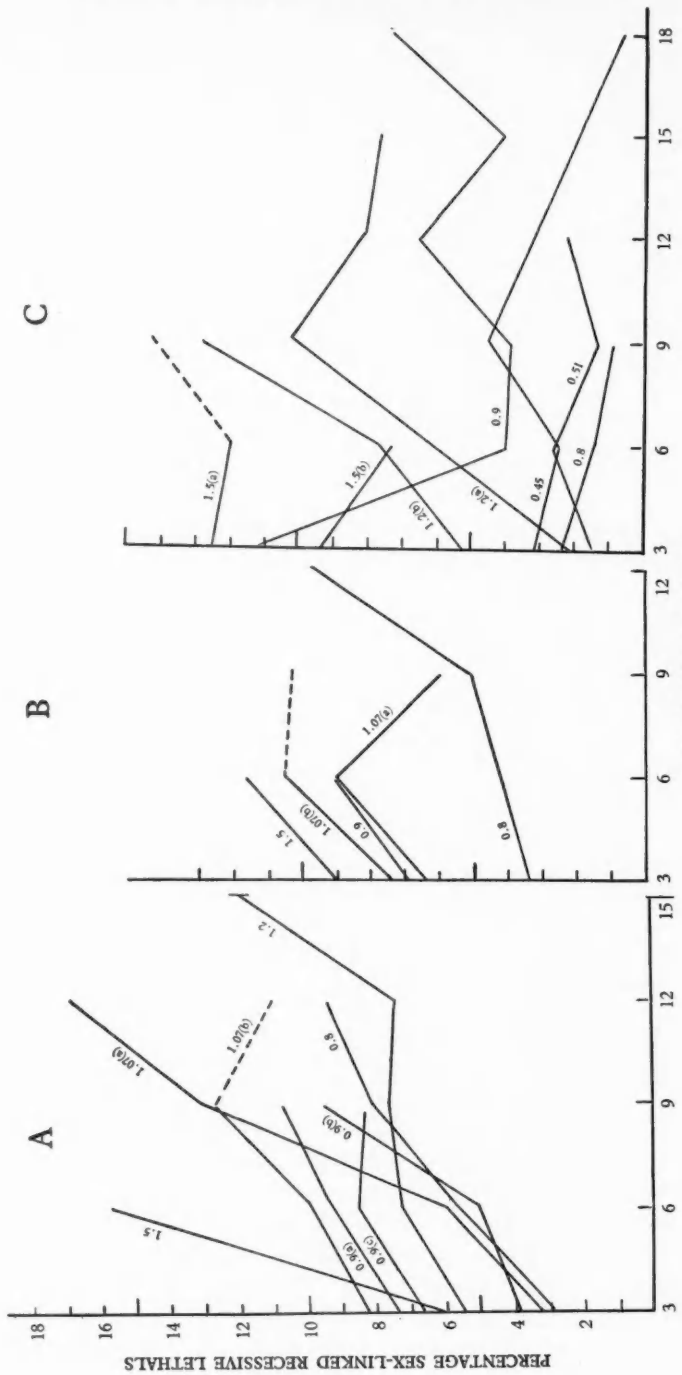

Fig. 1. The brood-mutation curves for the mustard derivatives of phenylalanine: A, the L-isomer, B, the D-isomer, and C the DL-mixture. The injected concentrations ($\times 10^{-2}$ M) are indicated for the separate experiments.

each pair of consecutive broods in the same experiment was compared in a 2×2 contingency table and a χ^2 for the significance of the deviation was calculated. The primary interest in this analysis, however, is to determine whether the deviation in the mutation rate for any two consecutive broods is in a common direction in experiments with different doses. The significance level P for each pair of broods in each experiment, therefore, should be based on one tail only of the corresponding distribution. As was shown by Yates (1955), χ (i.e. $\sqrt{\chi^2}$) for the individual tables, without correction for continuity, gives a good approximation to a normal deviate corresponding to the probability for a single tail. Yates has further pointed out that the values of χ represent deviations with unit standard deviation, and their sum is a normal deviate with a standard deviation $= \sqrt{n}$, where n is the number of estimations. This would, therefore, provide a means for testing association from a set of 2×2 tables, as occurs in the present study. The significance levels for the pairs of consecutive broods for the L-isomer reveal a decisive brood-mutation pattern. The mutation rate rises sharply from the first to the third brood, and is maintained at a high level in the later progeny. This pattern, however, is not clearly revealed by the

Table 5. *Sex-linked recessive lethals induced by the mustard derivatives of phenoxyphenylalanine (CB. 3051) and phenylamino-butyric acid (CB. 1385)*

Mutagen and concentration ($\times 10^{-2}$M)		Broods No.	Broods Days	Chromosomes	Lethals No.	Lethals %
CB. 3051						
1·2		I	0–3	635	14	2·2
		II	4–6	621	16	2·6
		III	7–9	720	23	3·2
		IV	10–12	83	1	1·2
		Total		2059	54	2·6
CB. 1385						
0·3		I	0–3	273	4	1·5
0·5		I	0–3	258	5	1·9
0·7	(*a*)	I	0–3	189	3	1·6
	(*b*)	I	0–3	219	6	2·7
1·0	(*a*)	I	0–3	112	4	3·6
	(*b*)	I	0–3	95	4	4·2

analysis of the data for the D, and DL forms. Nevertheless, here again there is clear evidence that the mutation rate in the later broods is of roughly the same order as in the earlier ones.

An attempt at the analysis of the brood-mutation relationship was also undertaken with two other amino-acid mustards (Table 5, and Purdom, 1957), viz. the derivatives of phenoxyphenylalanine, CB. 3051, and aminobutyric acid: CB. 1385. This analysis, however, was seriously handicapped by the severe toxic and sterilizing effects these agents exert on the treated males. With the aminobutyric acid derivative, no treated males survived more than 3 days, thus only permitting the determination of the mutagenicity on the mature sperm. With the phenoxyphenylalanine

derivative, toxicity was not so drastic, but infertility was pronounced as early as the third brood, and was very severe at the fourth and later broods. It was, therefore, often difficult to recover sufficient offspring for a firm determination of the mutation rate among sperm derived from the earlier germ-cells. Within the analysable first three broods, however, it was clear that under the phenoxy derivative, as with the phenylalanine mustard, the mutation rate tends to rise the later is the brood (Table 5). It is also of interest that in one of Purdom's experiments (*loc. cit.*, fig. 8, table 8), where it was possible to recover and test a reasonable number of sperm derived from

Table 6. *Sex-linked recessive lethals induced by the mustard derivatives of various phenylcarboxylic acids*

Mutagen and concentration ($\times 10^{-3}$M)		Brood No.*	Chromosomes	Lethals No.	Lethals %
CB. 1332	0·3	I	356	4	1·1
		II	378	6	1·6
		III	259	6	2·3
		IV	112	1	0·9
		Total	1105	17	1·5
	1·2	I	294	6	2·0
	1·6	I	258	10	3·9
CB. 1356	0·5	I	387	8	2·1
		II	412	6	1·5
		III	376	12	3·2
		IV	329	3	0·9
		Total	1504	29	1·9
	0·7	I	317	11	3·5
		II	324	14	4·3
		III	287	25	8·7
		IV	292	2	0·7
		Total	1220	52	4·3
	1·0	I	289	13	4·5
		II	282	7	2·5
		III	292	28	9·6
		IV	276	3	1·1
		V	266	—	—
		VI	301	1	0·3
		Total	1706	52	3·0
CB. 1331	0·3	I	458	13	2·8
		II	162	7	4·3
		Total	620	20	3·2
	0·7 (*a*)	I	358	14	3·6
	(*b*)	I	261	12	4·6
	1·0	I	309	20	6·5
CB. 1348	0·2	I	424	12	2·8
		II	467	16	3·4
		III	398	17	4·3
		IV	376	5	1·3
		Total	1665	50	3·0
	0·6 (*a*)	I	361	19	5·3
		II	354	21	5·9
		III	215	21	9·8
		IV	198	2	0·8
		Total	1128	63	5·6
	(*b*)	I	249	14	5·6
		II	215	19	8·8
		III	139	15	10·8
		IV	50	—	—
		Total	653	48	7·4
	1·0	I	267	33	12·4
		II	186	26	14·0
		Total	453	59	13·0

*Broods I–VI start, respectively, on days 0, 4, 7, 10, 13, 16.

treated spermatogonia (occurring in the seventh brood), a mutation rate of the same order as that occurring in the first three broods was obtained. This is so in spite of the fact that the mutation rate in a brood as late as the seventh could easily have been depressed by germinal selection. It would seem highly probable, therefore, that the brood-mutation relationship for the phenoxyphenylalanine mustard is of essentially the same type as that for the phenylalanine derivative.

2. The carboxylic-acid mustards

Experiments were undertaken to test the mutagenic variation in the successive broods of males treated with the mustard derivatives of homologous phenylcarboxylic acids. The detailed data for the separate compounds are given in Table 6 and

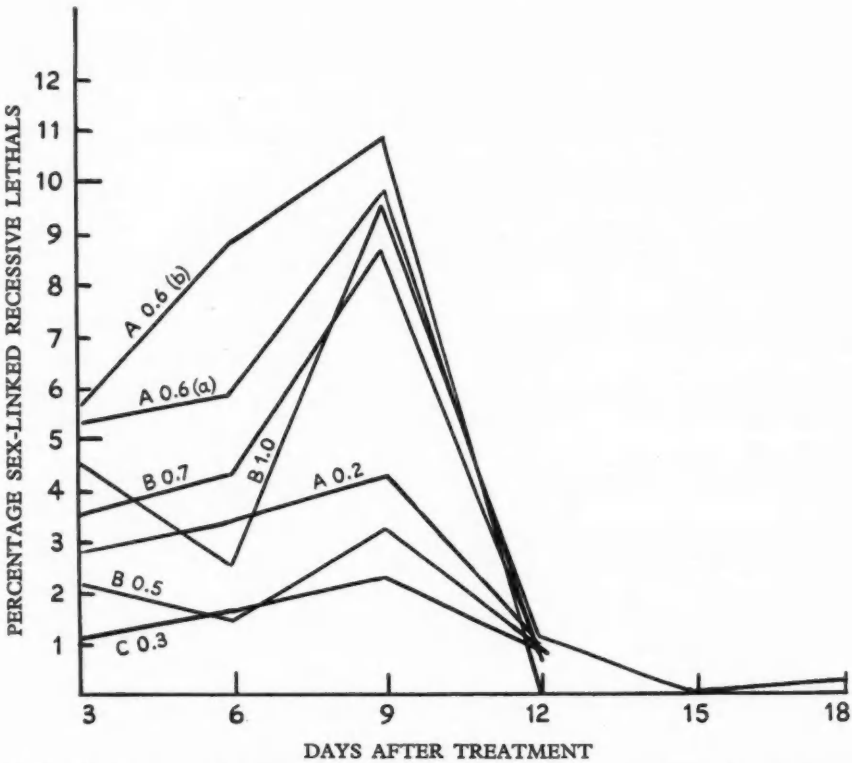

Fig 2. The brood-mutation curves for the mustard derivatives of various phenylcarboxylic acids: A, CB.1348; B, CB.1356; C, CB.1332. The injected concentrations ($\times 10^{-2}$ M) are indicated for the various experiments.

Fig. 2. In all experiments the mutation rate was high for the first three broods and then dropped to a very low level in the fourth. Furthermore, when the progeny fractionation was extended beyond this brood only the control mutation rate was recovered. It is certain, therefore, that compounds of this series are inactive on the germ-cells supplying sperm for the later progeny. The genetic analysis, therefore, was confined to the first four broods, 10–12 days after treatment. In some instances, however, and particularly at the higher doses, it was only possible to test one or two broods, as the treatment resulted in the partial sterilization and early death of the males.

The brood-mutation fluctuations for the various compounds tested have been analysed statistically by the χ method and the results are given in Table 7. A general

fluctuation pattern is discernible, and is best illustrated in experiments with the butyric and valeric acid derivatives (CB. 1348, 1356). Roughly the same mutation rate occurs in the first two broods, and this is followed by a sharp rise to a peak in the third brood, and then a drastic drop at the fourth brood, almost reaching the control level.

Table 7. *Distribution of χ for the increase in mutation rate for consecutive broods under the mustard derivatives of various phenylcarboxylic acids*

	Broods		
	I–II	II–III	III–IV
CB. 1331			
Total χ	0·9179		
No. of estimations	1		
P	0·18		
CB. 1332			
Total χ	0·5417	0·6650	
No. of estimations	1	1	
P	0·29	0·25	
CB. 1348			
Total χ	2·7214	2·9533	−6·3386
No. of estimations	4	3	2
P	0·09	0·04	0·000004
CB. 1356			
Total χ	−1·4090	7·4019	−11·1297
No. of estimations	3	3	3
P	0·21	0·00001	$< 10^{-9}$

3. *The phenylethylamine mustard*

From the previous two sections it is clear that a difference in the brood-mutation curves occurs with the carboxylic, as compared to the amino-acid, mustards. The mutation rate remains at a high level in sperm recovered later than the ninth day after treatment with the amino-acid mustards, while it drops drastically and consistently after the same period under the carboxylic acid mustards. In order to assess how far this is a function of the presence of an amino ($—NH_2$) group in the molecule, the mutagenicity of the mustard derivative of an aromatic amine (CB. 3034: *p*-NN-di(2-chloroethyl)aminophenylethylamine) was tested. This is the amine corresponding to the phenylalanine mustard, but devoid of the carboxyl group.

The brood-mutation relationship for this amine mustard is given in Table 8 and Fig. 3, and the statistical analysis of the significance of the fluctuations is given in Table 9. The overall data show that there is roughly the same mutation rate in the first two broods, leading to a significant rise in the third, a gradual though insignificant fall through the fourth and fifth broods, which is followed by a very drastic fall in later broods. It is clear, therefore, that the presence of an amino-group in the mustard-molecule results in an increase of the mutagenic response of some of the

Table 8. *Sex-linked recessive lethals induced by the mustard derivative of phenylethylamine*

Conc. ($\times 10^{-2}$M)	Brood No.*	Chromosomes	Lethals No.	Lethals %
0·19	I	417	17	4·1
	II	514	12	2·3
	III	485	31	6·4
	IV	507	31	6·1
	V	495	12	2·4
	VI	504	2	0·4
	VII	346	0	0·0
	Total	3268	105	3·2
0·29	I	388	16	4·1
	II	524	22	4·2
	III	488	28	5·7
	IV	486	29	6·0
	V	535	29	5·4
	VI	514	6	1·2
	VII	336	0	0·0
	Total	3271	130	4·0
0·38	I	419	29	6·9
	II	553	46	8·3
	III	64	3	4·7
	IV	75	7	9·3
	V	8	2	—
	VI	377	2	0·5
	VII	164	0	0·0
	Total	1660	89	5·4
0·77	I	62	8	12·9
	II	139	15	10·8
	III	9	—	—
	IV	1	1	—
	Total	211	24	11·4
1·15	I	43	7	(16·3)
	II	24	5	(20·8)
	III	—	—	—
	IV	1	1	—
	Total	68	13	19·1

*Broods I–VII start, respectively, on days 0, 4, 7, 10, 13, 16, 19.

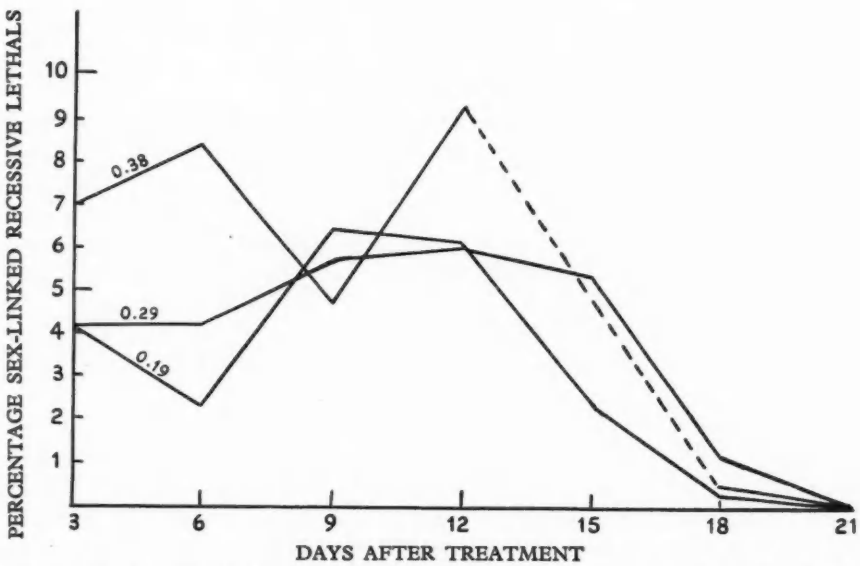

Fig. 3. The brood-mutation curves for the mustard derivative of phenylethylamine. The injected concentrations ($\times 10^{-2}$ M) are indicated for the separate experiments.

early germ cells. The drastic and consistent fall in the mutation rate later than the fifteenth day after treatment, however, shows that the earliest stages of the male germ-line are refractory to the amine mustard. In contrast, the amino-acid mustards did possess decisive mutagenic effect on the very young germ cells: recovered later than the fifteenth day after treatment (Tables 1 and 3). It would follow, therefore, that the response of these early germ-cells to the amino-acid mustard is not entirely a function of the presence of an amino-group in the mutagen.

Table 9. *The distribution of χ for the increase in mutation rate between consecutive broods under the mustard derivative of phenylethylamine*

	Broods				
	I–II	II–III	III–IV	IV–V	V–VI
Total	−0·6275	3·1254	−1·0863	−1·9017	−13·3162
No. of estimations	5	3	3	3	3
P	0·40	0·04	0·26	0·14	$< 10^{-9}$

IV. DISCUSSION

The interpretation of the brood-mutation curves in terms of cell-stage response is by necessity only inferential, and is bound to be very approximate. The germ line presents an uninterrupted spectrum of differentiation, and any attempt at its fractionation into separate cell stages by repeated matings can never be absolute. As was demonstrated in our work with TEM (2:4:6-tri(ethyleneimino)-1:3:5-triazine, Fahmy & Fahmy, 1954 and 1955), even under standard experimental conditions (as regards culture conditions, age of male and brood interval), alterations in the brood-mutation curves did occur, mainly due to cell killing and variation in speed of germ line differentiation. Nevertheless, through extensive histo-genetic studies (Fahmy & Fahmy, 1954), it was possible to show that in our experiments with TEM, post-meiotic stages of the testis are mainly utilized within the first four broods (i.e. 0–12 days after treatment), and the meiotic and pre-meiotic stages are utilized in later broods. Evidence is now available that this timing is approximately applicable to the majority of the alkylating mutagens tested in our laboratory. With 2-chloroethyl methanesulphonate (Fahmy & Fahmy, 1956 and 1957*a*) which is strongly mutagenic on the early germ-cells, 'clusters' of identical visibles were detected in the fifth brood (13–15 days after treatment), indicating that at least some of the sperm utilized in this period was derived from treated spermatogonia. In our study of the visibles induced in the present experiments with the phenylalanine mustard (Fahmy & Fahmy, 1959) a large sample of visibles was recovered in the fourth brood, but no 'clusters' were detected. In the fifth brood, however, though fewer mutations were recovered (because of sterility through cytotoxicity), visible clusters did occur. Most significant also are our results with S-chloroethyl cysteine (Fahmy & Fahmy, 1957*b* and under publication) which exerts specific mutagenicity on the early germ-cells. Here again clusters of identical sex-linked visibles occurred in the fifth brood, indicating that the sperm producing this progeny

came from treated spermatogonia capable of mitotic proliferation. Independent evidence pointing to the same conclusion came from the analysis of the time of onset of germinal selection. The study of the X-chromosome mutations revealed a disturbance in the visible to lethal ratio (in favour of the visibles) at the fifth brood, simultaneous with the appearance of the visible clusters. This suggested that germinal selection did operate against the recovery of a proportion of the lethals induced in proliferating spermatogonia. A comparative study of the autosomal and sex-linked lethals induced in the early germ-cells by the cysteine mustard, also showed that selection against the X-chromosome hemizygous mutations is first noticeable in the fifth brood. This confirms the timing of the onset of germinal selection deduced on the basis of the sex-linked mutations. Furthermore, it indicates that proliferating spermatogonia (supplying the hemizygous lethals against which selection operates) must also contribute to the sperm utilized in the fifth brood. There is little doubt, therefore, that under our experimental conditions, and with the most diverse alkylating agents, some of the sperm derived from treated spermatogonia starts to be utilized 13–15 days after treatment. This reasonable consistency in the separation of the spermatogonial from the post-spermatogonial stages probably indicates that under moderate doses of the alkylating mutagens the speed of the male germ-line differentiation is not drastically disturbed. It is highly improbable, however, that even this gross separation is achieved in every progeny fractionation experiment. In some instances the utilization of spermatogonia may begin as early as the fourth, or may be delayed until after the fifth brood.

In the light of the above considerations, it would be possible to give a broad interpretation of the brood-mutation curves for the compounds analysed in terms of cell-stage response. Under the carboxylic acid mustards, there is a peak of maximal mutability in the third brood followed by a sharp fall to almost the control level in the fourth and later broods. These compounds, therefore, give maximal activity on a post-meiotic stage, most probably an early spermatid. In this respect, it would appear that their mode of action is fundamentally comparable to X-radiation (Glass's 1956 review, and Fahmy & Fahmy—unpublished), as well as a diversity of chemical mutagens (e.g., mustard gas: Auerbach, 1950; diepoxybutane: Bird & Fahmy, 1953; and TEM: Fahmy & Fahmy, 1955). In contrast, under the amino-acid mustards, the overall trend is towards a rise in the mutation rate until the third brood, and the maintenance of the high rate in later progeny. This indicates that the later stages of spermatogenesis (i.e. the sperm and later spermatids) are less responsive to this series than the earlier stages (early spermatids, spermatocytes and spermatogonia). The brood-mutation curve for the amine mustard is nearer that for the amino-acid, than the carboxylic-acid mustards. The mutation rate reaches its maximum at the third brood, and is maintained at a high level through the fourth and fifth, but falls decisively to the control level in later broods. The earliest stages of the male germ line, therefore, seem to be refractory to the mutagenic action of the amine mustard. It is difficult to be certain, however, if this refractory stage represents the whole of the spermatogonial population, or only the earlier stages (i.e. the primary spermatogonia). It is perhaps of significance that no visible

'clusters' were detected in the fifth brood with the amine mustard. This may suggest that in this series of experiments the recovery of sperm from spermatogonia may have been delayed, in part at least, until the sixth brood. On this basis, it would appear that practically all the spermatogonia are refractory to the mutagenicity of the amine mustard.

The most significant fact that emerges from the present analysis is that while the amino-acid derivatives possess decisive mutagenic activity on the spermatocytes and spermatogonia, the carboxylic-acid mustards are practically ineffective on these cells. This does not seem to be entirely due to the mere presence of an amino (NH_2) group in the mutagenic molecule, since the phenylethylamine mustard is still inactive on spermatogonia (at least the earlier stages). It would seem, therefore, that the response of the gonia is a feature of the whole amino-acid moeity of the mutagen. Of interest in this connexion are the biochemical results (Cohn, 1957) that ^{14}C-labelled phenylalanine mustard, and its hydrolysates, become bound *in vivo* with the protein cell fractions of metabolically active tissues (rat liver and kidney). This binding is probably physical (through adsorption) rather than due to chemical incorporation (through a peptide linkage), since it has now been shown (Brookes, 1959) that the labelled pure dihydroxy-derivative (*p*-di-(2-hydroxy-$^{14}C_2$-ethyl)-amino-L-phenylalanine) is not incorporated in the protein fraction of metabolizing cells. Of all the stages of spermatogenesis, it is only the spermatocytes and the spermatogonia which are endowed with any anabolic activity. The mutagenic response of these cells to the amino-acid mustards, suggests some intracellular 'transport' mechanism associated with protein synthesis. This may proceed along the same pathway as that ensuring the supply of the unsubstituted amino-acid for nucleoprotein synthesis during chromosome replication, but without leading to the successful incorporation of the 'unnatural' molecule. The outcome of this transport mechanism, as regards mutagenesis, would be the conveyance of the reactive molecule through the cytosome to the hereditary nucleoproteins during chromosome reproduction. This could lead to a higher frequency of 'encounters' between the active groups of the mutagen and the genes, and would account for the observed mutagenic response.

SUMMARY

The analysis of the variation in the mutation rate in the fractionated progeny of treated males, revealed a marked differential cell stage response to the various chemical series investigated. The mustard derivatives of amino-acids (particularly L-phenylalanine) exert their minimal mutagenicity on mature sperm, but possess an appreciable activity on other stages of spermatogenesis, including spermatogonia. The carboxylic-acid mustards produce their maximal effect on an early spermatid, but are practically ineffective on spermatocytes and spermatogonia. The amine mustard corresponding to the phenylalanine derivative is effective on the stages of spermiogenesis (including the early spermatids) as well as on the spermatocytes, but is inactive on the spermatogonia (at least the primary stages). The response of the

gonia, therefore, is a function of the amino-acid moeity of the mutagen, and is not merely due to the presence of an amino-group in the molecule.

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Zigzag: a genetic defect of the horizontal canals in the mouse

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The name 'zigzag' has been given to a genetic behaviour defect in the house mouse (*Mus musculus* L.) in which the animals walk in a zigzag path. It arose spontaneously in a non-inbred strain of mice. The genetic studies reported in this paper have shown that the zigzag character is not due to a simple single gene change, but the anatomical studies have shown that the inner-ear defect responsible for the behaviour defect is different from all others previously reported, and for that reason it has been thought worth while to describe zigzag.

GENETICS OF THE 'ZIGZAG' CONDITION

Breeding within the stock in which zigzag animals were first found resulted in the production of some zigzag and some normal young from matings of both normal by normal and zigzag by zigzag (Table 1). This ruled out the possibility of the zigzag condition being due to a single gene with good penetrance, whether dominant or recessive.

Table 1. *Results of matings in the original zigzag stock*

	Offspring		
Parents	Zigzag	Normal	% Zigzag
Normal × Normal	14	54	20·2
Zigzag × Normal	30	174	14·7
Zigzag × Zigzag	22	74	22·9

To find whether the character behaved primarily as a recessive or as a dominant, zigzag animals were outcrossed to various stocks. With two of the outcross stocks, C57BL and YX, a few zigzag animals were observed in the F_1 generation (Table 2),

Table 2. *Results of outcrosses of zigzag animals from the original zigzag stock to unrelated stocks*

		Offspring	
Outcross stock	No. of pairs	Zigzag	Normal
CBA	5	0	54
C57BL	8	20	106
A	3	0	26
YX	5	3	82

and with the other two stocks no zigzags were found. This showed either that the zigzag character was the result of the action of a single dominant gene with very low penetrance, or else that the inheritance was polygenic with some normal stocks having a higher level of zigzag polygenes than others. To test these possibilities a stock giving a high frequency of zigzag was built up by selection, and then the breeding behaviour of the zigzag and normal young from this stock was studied. If

Table 3. *Results of selection for increased frequency of zigzag young in matings of zigzag × zigzag*

Generation	No. of pairs	Offspring		
		Zigzag	Normal	% Zigzag
1	2	13	33	28·3
2	5	28	28	50·0
3	8	73	71	50·7
4	2	18	8	69·2
5	6	48	35	57·8
6	6	44	17	72·1
7	4	35	11	76·1

$\chi^2_{[1]} = 27{\cdot}6 \qquad P < 0{\cdot}001$

zigzag were due to a single dominant gene whose penetrance had been improved by the selection, then non-zigzags from the selected stock should not carry the gene and should not throw zigzag young. The method of selection was to keep young for breeding from those pairs in any generation which threw the highest proportion of zigzag young; brother-sister mating was avoided. Table 3 gives the results of the

Table 4. *Results of test matings of zigzag and normal young from the zigzag stock after selection for high frequency of zigzag*

Parents		No. of pairs	Offspring		
♀	♂		Zigzag	Normal	% Zigzag
Zigzag ×	Zigzag	10	173	62	73·6
Zigzag ×	Normal	5	48	20	71·1
Normal ×	Zigzag	5	43	17	
Normal ×	Normal	4	50	76	39·7

selection and Table 4 of the test-matings which followed it. A χ^2 method due to Holt (1948) with a computational simplification by Dr B. Woolf was used to test the statistical significance of the apparent progress made by selection. Let a be the generation of selection; let A_1 and A_2 be $S(a)$ for all zigzag and normal young respectively; and let n_1 and n_2 be the total numbers of zigzag and normal young. Then

$$\chi^2 = \frac{(n_2 A_1 - n_1 A_2)^2}{n_1 n_2 S(a-\bar{a})^2}.$$

Although the number of pairs used in the selection experiment was very low it is clear that significant progress was in fact made. The proportion of zigzags in the sixth and seventh generations is consistent with the zigzag character being due to

a single dominant gene for which the parents were heterozygous. The results of the test-matings show, however, that there was little difference in breeding behaviour between the normal and zigzag animals from the selected stock. Matings of zigzag × normal threw as high a proportion of zigzag young as matings of zigzag × zigzag, which gave results like those of the last generations of selection. The proportion of zigzag young from matings of normal × normal was lower, but all four pairs threw some zigzag animals, i.e. there was no reason to suppose that there was a single gene for zigzag which some pairs did not carry. Thus, the hypothesis that the zigzag character is the result of the action of a single dominant gene with modifiers is not supported by these data. There remains the alternative explanation of polygenic inheritance. In an attempt to obtain some idea of the number of polygenes involved zigzag animals from the selected stock were outcrossed and the young then intercrossed to obtain an F_2. Only about 1 in 50 F_2 animals was affected

Table 5. *Results of the outcross and following intercross of animals from the selected stock*

	No. of pairs	Offspring	
		Zigzag	Total
Outcross	2	1	69
Intercross	8	8	443

(Table 5). This is consistent with the 1 in 64 expected if zigzag were due to the simultaneous homozygosis of three unlinked recessive genes, but obviously many other polygenic situations are possible.

THE BEHAVIOUR DEFECT OF ZIGZAG MICE

The most usual characteristic of the zigzag mice was a zigzag motion of the head in walking and a tendency to describe a complete circle now and then. There was no vertical head-shaking; the animals showed normal responses to change of position and a normal 'landing reaction', and their hearing was normal. The expression of the defect varied. The most severely affected animals circled repeatedly, but at the lower end of the scale the zigzag head motion was sometimes very slight with no circling, so that the defect graded into normal.

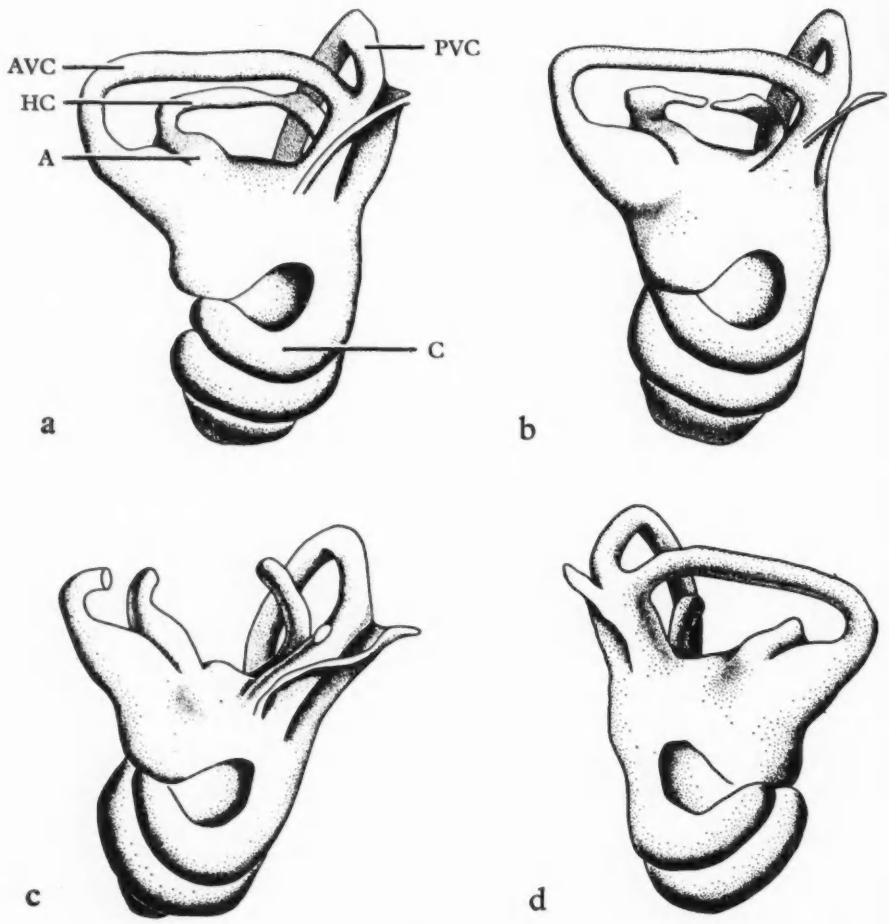
STUDIES OF THE INNER EAR DEFECT

Whole mounts of the bony labyrinth and sections of the inner ear were prepared by the methods of Lyon (1958).

The whole mounts showed that in zigzag animals there was either reduction or absence of one or both horizontal canals (Fig. 1). There were all grades of defect from complete absence of the canal to normality; in some animals one ear was normal and the other abnormal. Intermediate grades did not consist of shortening of the canal but of a constriction in the middle of its length. Sometimes this constriction was a simple narrowing, the narrow part varying in length from a mere

notch to about one-third of the canal length. Sometimes the middle portion of the canal was lacking, leaving blind-ending stumps at both ends. The stumps again varied in length according to the severity of the defect, but even in the most severe grades the ampulla of the canal was always present.

Sections of the inner ear revealed no additional abnormalities. The crista and ampulla of the horizontal canal were normal, as also was the remainder of the inner



Text-fig. 1. Camera lucida drawings of whole mounts of the bony labyrinths of zigzag mice, showing increasing grade of defect from a slight constriction of the horizontal canal in *a* to almost complete absence of the canal in *d*. *a–c* are from right ears and *d* is from a left ear. In *c* the anterior vertical canal has been cut away to give a better view of the horizontal canal. A = ampulla of the horizontal canal, AVC = anterior vertical canal, C = cochlea, HC = horizontal canal, PVC = posterior vertical canal.

ear. The stumps of membranous canal ended blindly where the bony canal ended, or if the bony canal were merely constricted then a thin shred of tissue with no canal lumen passed through the constriction.

Of 22 zigzag animals examined, 18 had some abnormality of the horizontal canals of both ears and 4 had one ear normal and one abnormal. The ears of 11 apparently normal animals from the zigzag stock were also examined; 6 had both ears normal, 4 had one ear with some abnormality (merely a constriction in three cases), and 1 animal had both ears abnormal. Thus the correlation between ear defect and behaviour defect was good, but the one anomalous animal with abnormal ears and normal behaviour showed that the ear defects were not always incompatible with normal function of the ear.

COMPARISON WITH OTHER MUTANTS

Many mutants affecting the inner ear of the mouse are already known (Grüneberg, 1956) and they can be broadly divided into those in which the ear is morphologically normal but there are degenerative nerve changes, and those involving morphological defects of the ear. Zigzag clearly belongs to the second group, the defects of some of which are tabulated in Table 6. Other mutants affecting chiefly the canals are

Table 6. *The defects of some mutants with morphological effects on the inner ear*

Ear Defect				Behaviour		
Otoliths	Horizontal canal	Vertical canals	Mutant	Position response	Horizontal movement	Vertical movement
A	N	N	Pallid	A	N	N
N	A	N	Zigzag	N	A	N
N	A	A	Fidget	N	A	N
N or A	A	N or A	Twirler	N or A	A	N or A
N or A	A	A	Dreher	N or A	A	A
A	A	A	Kreisler	A	A	A

A = abnormal, N = normal

fidget and twirler. In fidgets all the canals are rudimentary (Truslove, 1956), and in twirlers the horizontal canals are chiefly affected but they are shortened rather than constricted as in zigzags, and the ampullae are abnormal (Lyon, 1958).

In fact zigzag is the only genetic ear defect of the mouse so far described in which the canals are constricted rather than shortened. There is thus no reason to suspect that zigzag might be a recurrence of a previously known mutant, and no high probability of it being allelic with any. No direct tests of allelism have been made, but the results of linkage tests suggest that no zigzag factor is allelic with kreisler, pallid or fidget. All three of these genes lie in linkage group V, as also does the agouti locus. Some matings in the zigzag stock could have given evidence of linkage of a zigzag factor with agouti or with pallid; no evidence of such linkage was found (Table 7). Thus there is no reason to suppose that any zigzag factor lies in this

Table 7. *Linkage tests between zigzag and two markers in linkage group V*

Parents	Zigzag offspring: Observed +	Observed a	Expected* +	Expected* a
++/zga × ++/zga	6	2	6	2
++/zga × zga/zga	4	8	6	6
zg+/+a × zga/zga	2	6	4	4
	+	pa	+	pa
++/zgpa × ++/zgpa	5	1	4·5	1·5

* On the hypothesis of independent segregation.

linkage group. (All these matings happened to be ones which threw only a low proportion of zigzag young and therefore the linkage tests have been made simply by noting whether the segregation of agouti or pallid among the zigzag animals departed from that expected on the basis of independent segregation. The non-zigzag animals would yield very little linkage information as, on the hypothesis being tested, they must include a large proportion of the genetically zigzag animals as well as the true non-zigzags.) Tests with dreher and twirler have not been made.

DISCUSSION

The zigzag character does not show simple Mendelian inheritance. In this it resembles various other characters of the mouse, including hare-lip (Reed, 1936), duplicate incisors (Danforth, 1958) and white spotting (Dunn & Charles, 1937). The first question to be asked about such a character is whether it is determined by one major gene with modifiers or by several genes with equal effect. Wright (1934*a*, *b*), dealing with polydactyly in guinea-pigs, showed that the hypothesis of a major gene needs stringent test before it can be accepted. In the case of zigzag, simple breeding tests of normal and zigzag animals from the same stock showed no major genetic differences between them, and the results of outcrossing and intercrossing gave no indication of the segregation of a gene which could be described as 'major'. Thus zigzag is thought to be due to the action of genes which individually have only small effects. On crossing to different stocks, there were in some cases affected animals in the first generation and in some cases none. This suggests that the underlying developmental basis of the zigzag condition has a continuous distribution, the affected animals are those in which a certain threshold is passed, and the stocks concerned are genetically at different levels with respect to the threshold. The rapid progress which was made in the small selection experiment might appear to suggest that only a small number of genes were concerned, but this experiment started from a stock in which the level of the underlying basis must have been near the threshold for zigzag, since there were 28% affected animals in it. A different foundation stock might have resulted in very different progress. The hypothesis of a continuous underlying basis with a threshold for abnormality was first put forward

by Wright (1934*b*) to explain the inheritance of polydactyly in guinea-pigs and has since been suggested for various mouse characters, including absence of third molars (Grüneberg, 1951). It may thus be a common type of inheritance in mammals.

SUMMARY

The name zigzag has been given to an inherited behaviour defect in the mouse in which the animals walk with a zigzag motion. It is inherited polygenically. The anatomical defect responsible for the abnormal behaviour was a reduction or absence of the horizontal canals of the inner ear, the reduction consisting of a constriction in the middle of the canal length, rather than a shortening of the canal.

The author is grateful to Mrs M. L. Duffill for technical assistance and for drawing Fig. 1.

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Genetical studies on the skeleton of the mouse

XXV. THE DEVELOPMENT OF SYNDACTYLISM

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(*Received* 16 *September* 1959)

This paper is dedicated to my teacher and friend, Professor Hans Nachtsheim, on the occasion of his seventieth birthday.

INTRODUCTION

In an earlier paper of this series* (Grüneberg, 1956), an anatomical description of the osseous and cartilaginous skeletons has been given of the three mutants syndactylism (*sm*/*sm*), Oligosyndactylism (*Os*/+) and shaker with syndactylism (*sy*/*sy*) in the mouse; in each case, the description started with 14-day embryos in which the chondrification of the limb skeleton is sufficiently advanced for the application of bulk staining techniques (methylene blue). The anatomical situation encountered was quite distinct for each of the three mutant genes: evidently they owe their existence to different mechanisms of gene action. The embryological analysis of syndactylism to be presented in this paper shows that in this condition the skeletal system is only secondarily involved.

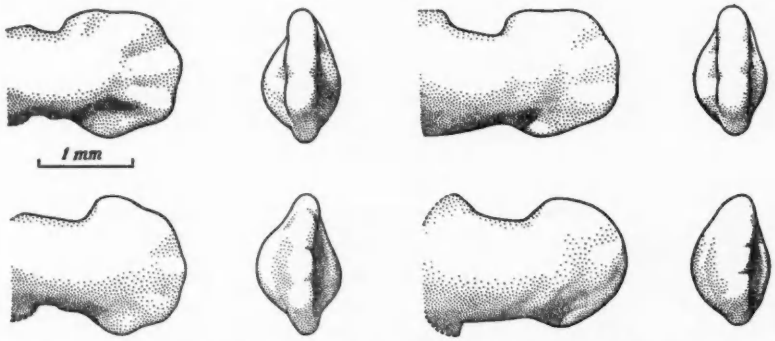
ADULT ANATOMY

As described in more detail previously (Grüneberg, 1956), the gene for syndactylism invariably affects all four feet. The third and fourth digits are always syndactylous; digit 2 is often and digit 1 occasionally involved. Digits of the fore feet are usually joined by soft tissues only, those of the hind feet generally by cartilage or bone. Fusions tend to involve all three phalanges, but not the metacarpals or metatarsals respectively. The fusions are always primary, i.e., corresponding phalanges of neighbouring digits are represented by a single cartilaginous element from the start. Secondary fusions between the os naviculare and the os cuboideum of the hind feet occurred in 8 out of 21 *sm*/*sm* mice and probably represent a remote gene effect; they will not be dealt with in this paper. Many *sm*/*sm* mice have tail kinks and occasionally a tail twist; these are confined to the distal half of the tail, with a strong preference for the third quarter (caudal vertebrae 16–21).

* The first 21 papers of this series, by the present author and by various other members and guests of this research group, have appeared in the *Journal of Genetics*, vols. 50–55, 1950–7; papers 22–24 have been published in the *Journal of Embryology and Experimental Morphology*, vol. 6, 1958.

EXTERNAL FEATURES OF SYNDACTYLOUS EMBRYOS

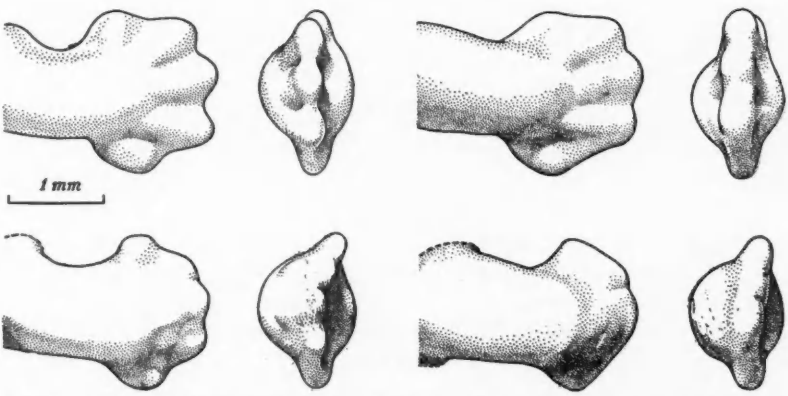
As *sm/sm* mice are fertile in both sexes, litters of embryos consisting entirely of *sm/sm* individuals can be compared with corresponding litters of normal embryos. This has been done for the younger stages where classification by external features



Text-fig. 1. The right fore and hind limbs of a normal (top row) and an *sm/sm* embryo. 12-day-old litter-mates; C.R.L. 7·8 and 8·0 mm. respectively. Camera lucida drawings.

is either uncertain or altogether impossible. From the 12-day stage onwards, classification by external inspection is easy and litter-mates from segregating litters have been compared with each other.

In litters of 10 and 11 days (mean C.R.L. 4·15 and 5·0 mm. respectively), the limb buds of *sm/sm* embryos do not appear to differ externally from those of normal embryos of comparable stages. By the 12-day stage (Text-fig. 1), a characteristic difference has made its appearance. The limb buds of *sm/sm* embryos appear bloated; the dorsal surfaces are curved more strongly than in normal embryos while



Text-fig. 2. The right fore and hind feet of a normal (top row) and an *sm/sm* embryo. 13-day-old litter-mates; C.R.L. 9·3 and 9·0 mm. respectively. Camera lucida drawings.

the palmar and plantar surfaces are flattened. As a result of this deformation, the free edge of the foot plates is bent over in a palmar and plantar direction respectively and the length of the circumference is shortened. Less space is thus available for the middle digits to spread out.

Similar but more extreme deformations of the feet are again found in 13-day *sm/sm* embryos (Text-fig. 2). By now the palma and planta have actually become concave and the middle digits are still more crowded together as shown by the indentations of the foot margin.

In older embryos, the bloatedness of hands and feet gradually diminishes as the digits grow out, with the result that the middle and terminal phalanges of basally syndactylous digits sometimes manage to differentiate as separate entities (Grüneberg, 1956; Figs. 5-7, p. 120).

In 12- and 13-day-old *sm/sm* embryos, the tail tip is often bent over backwards in a dorsal direction. There is much variation in this respect, and it may be assumed that the more strongly affected embryos would have shown tail kinks later in life.

SECTIONED SYNDACTYLOUS EMBRYOS

The sectioned material is summarized in Table 1. Six out of sixty-eight embryos were sectioned completely; in the remainder, only the limbs (and often the tails) were sectioned. All embryos of 15–12½ days and all but one pair of 12-day embryos came from segregating matings; the normals are thus largely $+/sm$ heterozygotes. One 12-day and all 11- and 10½-day normals are F_2 embryos from a cross between

Table 1. *Normal and syndactylous embryos sectioned*

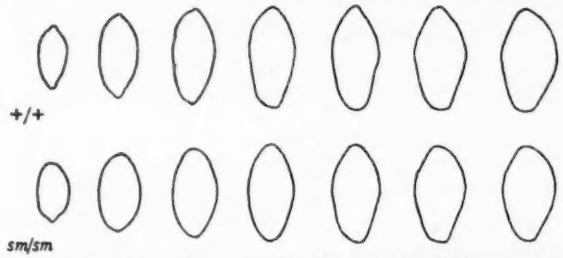
Age	Normal	*sm/sm*	Remarks
15	–	2	Palmar-plantar sections; no tails
14	2	4	1 trio palmar-plantar, 1 trio transverse sections; no tails
13	4	6	1 pair completely sectioned (transverse); 1 trio palmar-plantar and 1 trio transverse, both without tails; 1 pair transverse with tails
12½	2	2	Transverse sections, with tails
12	6	9	2 pairs completely sectioned (transverse); rest transverse sections, with tails
11	8	8	Fore limbs and tails transverse sections; hind limbs dorso-ventral sections
10½	7	8	Fore and hind limbs dorso-ventral sections; no tails
Total	29	39	

the inbred strains CBA/Gr and C57BL/Gr; they are thus $+/+$; one 12-day and all 11- and 10½-day *sm/sm* embryos come from matings of the type $sm/sm \times sm/sm$. The various abnormalities of *sm/sm* embryos will now be considered in turn.

1. *The external shape of the limbs*

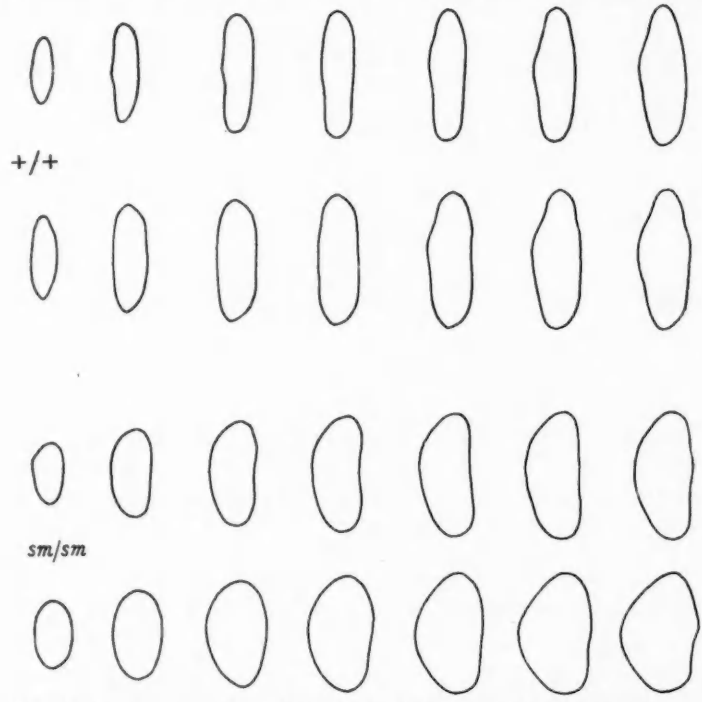
The findings are in close agreement with those from external inspection. No shape anomalies have been noticed in the limb buds of 10½-day-old *sm/sm* embryos. At that stage there is not yet any separation into foot plate and leg either in the fore

or in the hind limbs. At the 11-day stage, this separation has taken place in the fore limbs. In transverse sections of the fore limbs (Text-fig. 3), there is not yet much



Text-fig. 3. Transverse sections (7·5 μ thick) of the left fore limbs of a normal embryo (No. 1344; C.R.L. 4·9 mm; top row) and an *sm/sm* embryo (No. 1348; C.R.L. 5·1 mm; bottom row), 11 days old. In each case, the 10th, 20th . . . 70th sections as counted from the free margin are shown. Projection drawings made at magnification × 50; final magnification × 20. Same technique and magnifications in text-figs. 4–6.

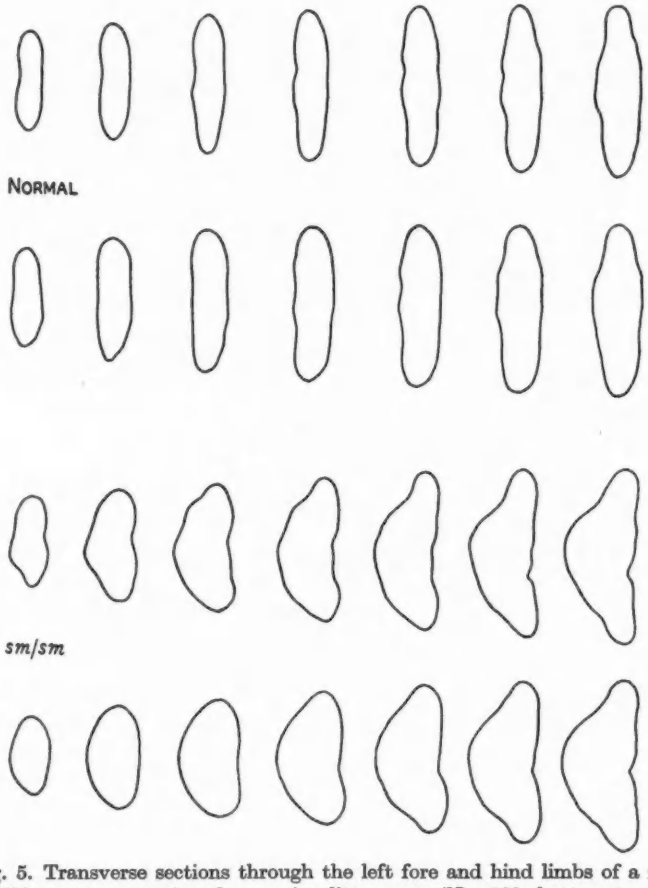
difference between normal and *sm/sm* embryos; perhaps the fore limbs are a little narrower but thicker than those of the normal embryos, but the difference is at most very slight. In the hind-limb buds (which are in the stage of semicircular rudders),



Text-fig. 4. Transverse sections through the left fore and hind limbs of a normal (No. 1070; C.R.L. 7·6 mm.; top two rows) and an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; bottom two rows), 12 days old.

there are no obvious shape differences (Figs. 6 and 7, Plate I), though, of course, slight differences might be detectable with a more elaborate technique.

In the 12-day stage (Text-fig. 4), striking differences have developed both in the fore and in the hind limbs. The limbs are narrower from side to side but much



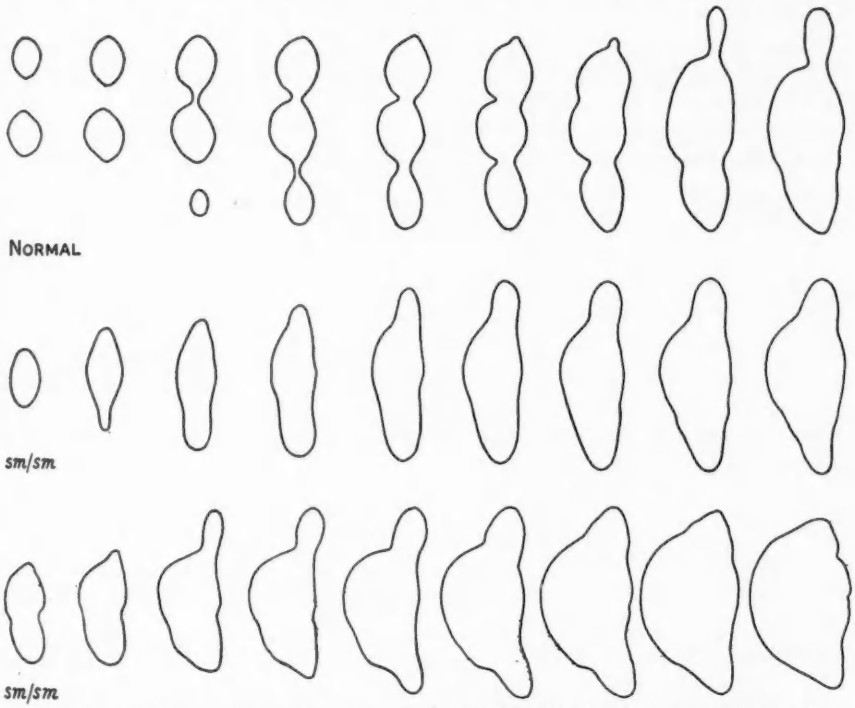
Text-fig. 5. Transverse sections through the left fore and hind limbs of a normal (No. 568; top two rows) and an *sm/sm* litter-mate (No. 569; bottom two rows), 13 days old; mean C.R.L. of litter 9·0 mm.

thicker in a dorso-ventral direction. The foot plates are blown up dorsally while the palmar and plantar surfaces are flatter than normal. The hind limbs are more strongly affected than the fore limbs.

The difference between normal and *sm/sm* limbs becomes even more marked in the 13-day stage (Text-fig. 5), with the palmar and plantar surfaces now actually concave. In the 14-day stage (Text-fig. 6), *sm/sm* embryos are more variable. In the first of the two syndactylous embryos shown, the limb is more nearly normal while in the second animal it is even more bloated than in the 13-day stage. How-

ever, as can be seen by external inspection of *sm/sm* embryos, in later stages there is a general tendency for the feet to return to more normal proportions.

Text-figs. 4–6 suggest that the foot plates of *sm/sm* embryos show a real increase in bulk and not merely an increase in thickness at the expense of width. This is



Text-fig. 6. Transverse sections through the left hind limbs of a normal (No. 571) and two *sm/sm* litter-mates (Nos. 572 and 573), 14 days old; mean C.R.L. of litter 10·3 mm.

borne out by planimetric measurements (Table 2). These show that the *sm/sm* foot plates are bulkier than those of normal embryos, and that the difference increases between the 12- and 13-day stage, at least in the fore limbs. The leg proximal to the foot plate is of about normal size. Sections show that the tissue density of the *sm/sm* foot plates is about the same as in the normal. Hence the increase in volume is not due to an oedema, but to an increase in cell number.

Table 2. *Sum of the areas of transverse sections* 10, 20, . . . 100 *in* cm^2 *in projection drawings* (× 50) *as determined by planimetry. Same animals as in Text-figs.* 4 *and* 5

	12 days		13 days	
	Fore limb	Hind limb	Fore limb	Hind limb
Normal (N)	54·1	61·1	66·4	75·3
Syndactylous (S)	62·8	78·5	95·5	98·2
S/N	1·16	1·28	1·44	1·30

2. *Invagination of the limb epidermis*

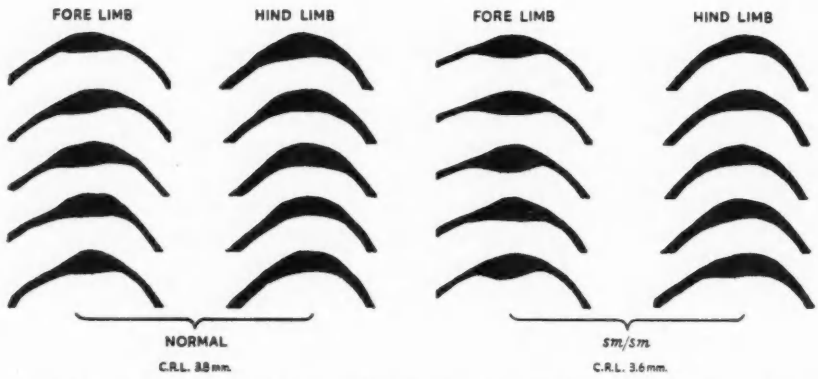
As the palmar and plantar surfaces of the foot plates become first flattened and in the end concave (i.e. from the 12-day stage onwards), folds of the epidermis of palma and planta sink into the underlying mesenchyme. Such invaginations often originate in the apical ectodermal ridge and may be visible as fine deepened lines in the intact limb (see, e.g., the end-on view of the hind limb in Text-fig. 2 above). A very slight ingrowth of this kind is shown in Fig. 10, Plate II. Sometimes such folds are not in contact with the apical ectodermal ridge, but start independently on palma or planta, like that shown in the fourth and fifth sections of Text-fig. 13 below. Commonly quite deep invaginations of epidermis may come to lie between the metacarpalia or metatarsalia, like the one shown in Fig. 11, Plate II. In transverse sections, such invaginated epidermal folds which originate in the apical ectodermal ridge are seen to reach across from the dorsal to the palmar or plantar surface respectively.

There can be no doubt that the invagination of skin-folds on the edge and on the palmar and plantar surfaces of the limbs is a mechanical consequence of the enlargement of the hands and feet which leads to an increase of curvature on the dorsal and a corresponding decrease of curvature on the opposite side. The epidermis is thus obviously passively pushed into the underlying mesenchyme by the forces which lead to the deformation of the limb.

3. *The apical ectodermal ridge*

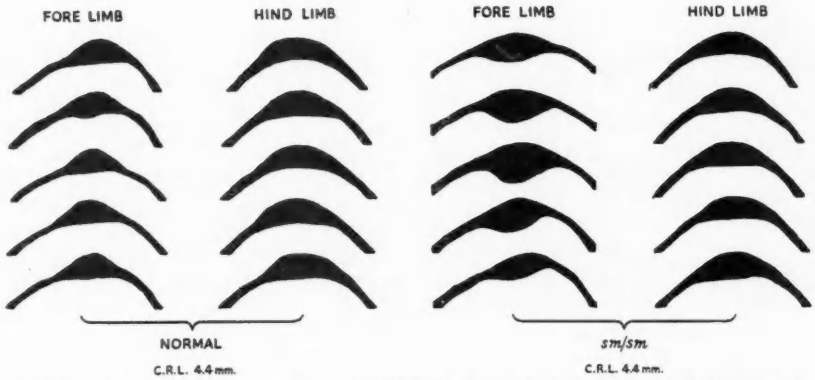
As will be discussed in more detail below, this structure plays an important part in the growth of the avian and mammalian limb buds. It is strikingly abnormal in *sm/sm* embryos.

In embryos of a developmental age of 10 days (Text-fig. 7), the apical ectodermal



Text-fig. 7. AER of the right fore and hind limbs of a normal (No. 1370) and an *sm/sm* embryo (No. 1361), developmental age 10 days. Dorso-ventral sections through the limb buds. In each case, the middle section of the five represents, as nearly as possible, the central section through the limb bud; it is flanked above and below by the fifth and by the tenth section from the middle either way; sections 7·5 μ thick. Projection drawings made at magnification × 250; final magnification × 100.

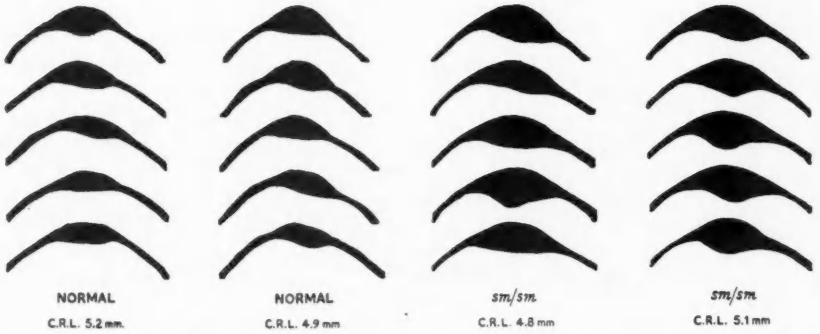
ridge (AER) of *sm/sm* embryos is still virtually normal as judged by its size. In the hind limbs there is no detectable difference. In the fore limbs, there is just a suggestion that the AER is a little thickened and projects convexly into the underlying mesenchyme; as later on this becomes a characteristic feature of *sm/sm* embryos, the



Text-fig. 8. AER of the right fore and hind limbs of a normal (No. 1364) and an *sm/sm* embryo (No. 1358), developmental age 10½ days though litter-mates of the embryos in Text-fig. 7; see there for further explanations.

small difference here encountered in the fore limbs can probably be accepted as an initial stage of the AER anomaly.

A slightly older stage is shown in Text-fig. 8 (actually, these two embryos belong to the same litters as those in Text-fig. 7, but represent a more advanced stage of

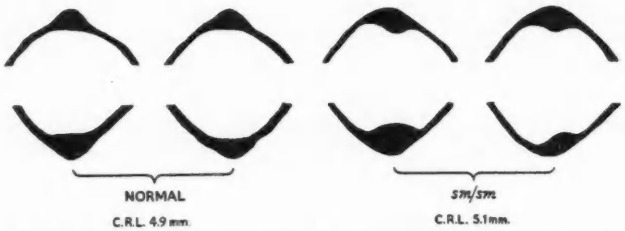


Text-fig. 9. The AER of the left hind limbs of two normal (Nos. 1343 and 1344) and of two *sm/sm* embryos (Nos. 1347 and 1348), 11 days old. For further explanations see Text-fig. 7.

development). While the AER of the hind limbs is still of approximately normal size, that of the fore limbs is now clearly thickened and projects deeply into the underlying mesenchyme. Whereas in sections the AER of the normal embryo projects nipple-like over the surface of the limb bud, that of the *sm/sm* embryo is embedded in the underlying mesenchyme rather than lying on top of it.

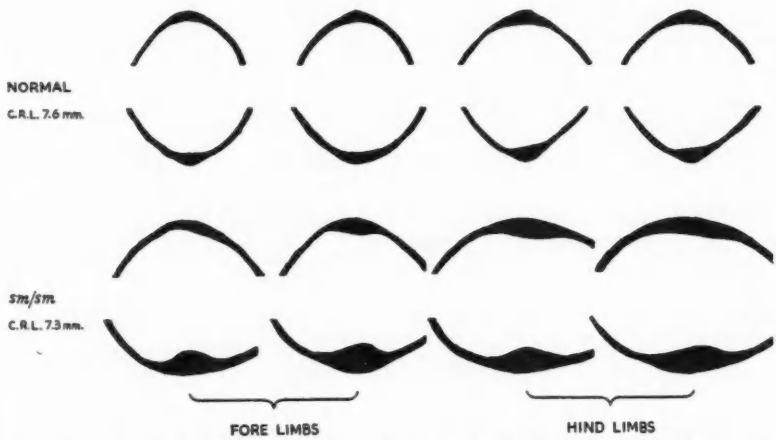
In the 11-day stage (Text-fig. 9), the AER of the hind-limb buds is also clearly thickened and much bulkier than that of the normal embryos. The difference in the AER of the fore limbs is still about as marked as it was in the 10½-day stage (Text-fig. 10).

In normal 12-day mouse embryos the AER has become much smaller; in the fore



Text-fig. 10. The AER of the left fore limbs of a normal (No. 1344) and an *sm/sm* embryo (No. 1348), 11 days old. Transverse sections; the AER is cut twice; in each case the 30th and 40th sections as counted from the free edge of the limb have been drawn. Projection drawings made at magnification × 250; final magnification × 100.

limbs it has almost disappeared whereas in the hind limbs it is still present as a definite though much smaller structure; here, as generally, the fore limb is ahead in development as compared with the hind limb. In 12-day *sm/sm* embryos (Text-

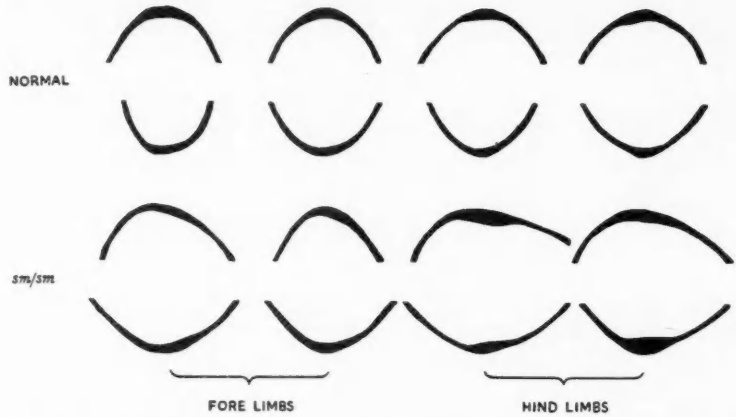


Text-fig. 11. The AER of the left fore and hind limbs of a normal (No. 1070) and an *sm/sm* embryo (No. 1071), 12 days old. Transverse sections. For further explanations see text-fig. 10.

fig. 11), the AER has also been reduced, but it still is much more massive, both in fore and hind limbs, than in the normal.

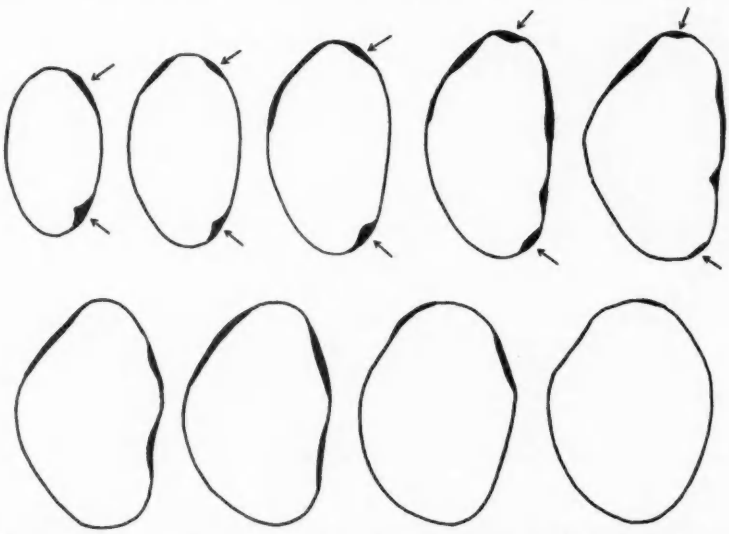
In normal 13-day mouse embryos, the AER has practically disappeared both anteriorly and posteriorly (Text-fig. 12). In *sm/sm* embryos of that age, this also

applies to the fore limbs where there is now no longer an appreciable difference. However, the AER of the hind limbs is still fairly conspicuous even at this late stage.



Text-fig. 12. The AER of the left fore and hind limbs of a normal (No. 568) and of an *sm/sm* litter mate (No. 569), 13 days old; mean C.R.L. of litter 9·0 mm. For further explanations see Text-fig. 10.

In a normal limb, the AER occupies a position almost exactly on the edge of the foot plate. In the bloated limbs of 12- and 13-day *sm/sm* embryos, the AER is regularly pulled in a palmar or plantar direction respectively (Text-fig. 13).



Text-fig. 13. Transverse sections through the left hind limb of a 12-day *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.). Sections 20, 30 . . . 100 as counted from the free edge of the limb. The AER is indicated by arrows. Projection drawings made at magnification × 80; final magnification × 40.

Microphotographs of the AER of normal and *sm/sm* embryos are given in Figs. 1–4, Plate I. No grossly pathological features have been noticed in the enlarged AER of *sm/sm* embryos in H. & E. preparations. However, there are certainly differences in structure between a normal and an *sm/sm* AER. Hence additional investigations with more refined (histochemical, etc.) techniques are desirable, and it is hoped that supplementary data will be given on a later occasion. In the meantime, as the enlargement of the AER is not merely due to an increase in cell size, but to an increase in cell number, it seems appropriate to speak of a hyperplasia of the apical ectodermal ridge.

4. *Hyperplasia of the limb epidermis*

The hyperplasia of the AER described above is not an isolated phenomenon. It is paralleled by events which take place in the epidermis of the limbs at about the same time.

The epidermis of the limbs of normal 10–12-day-old mouse embryos corresponds to Hanson's (1947) Stage B; i.e. it is two-layered with a more or less cylindrical stratum germinativum and a flat periderm whose cells are at first sparse. The normal appearance is shown in Figs. 6 and 8, Plate I. In *sm/sm* embryos, fairly sharply circumscribed areas of the limb epidermis, both on the fore and the hind limbs, become hyperplastic. The affected patches of epidermis are thickened and may ultimately be 4–5 cell layers deep in places. A typical localization is a dorsal area in the post-axial half of the limb which does not reach its free edge; it is shown, in an 11-day embryo, in Figs. 7 and 9, Plate I. A somewhat similar area is usually found on the ventral (palmar or plantar) aspect of the limb, but rather more preaxially. The distribution of hyperplastic areas on the left hind limb of a 12-day *sm/sm* embryo is shown in Text-fig. 13 above, and a microphotograph of a section through the same limb in Fig. 5, Plate I. The first signs of hyperplasia were seen in

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Explanation of Plates

All the material is fixed in Bouin's fluid, embedded by Peterfi's method, sectioned at 7·5 μ and stained with haematoxylin and eosin.

PLATE I

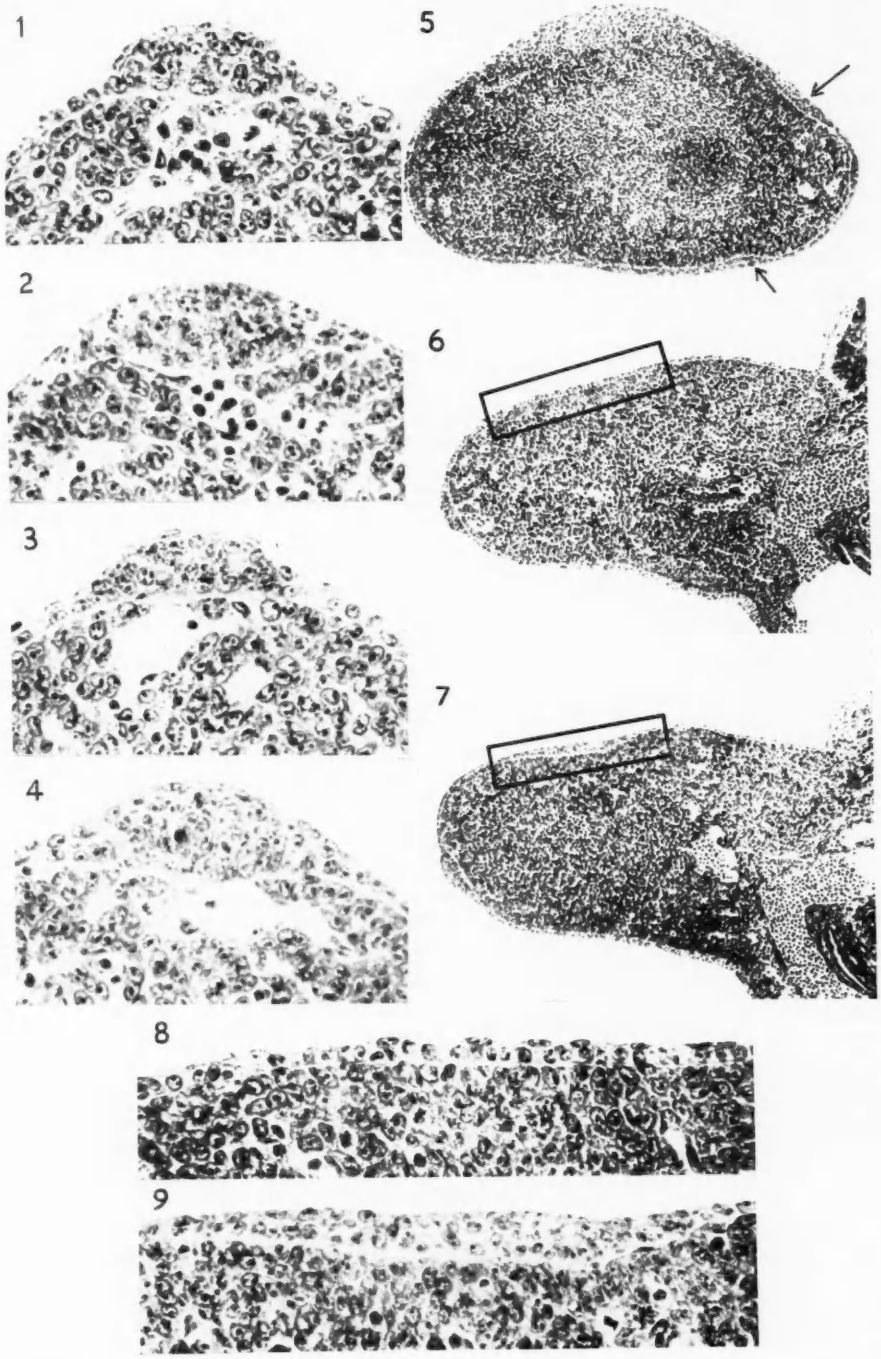
Figs. 1 and 2. The AER of the left fore limbs of a normal (No. 1364; C.R.L. 4·4 mm.) and of an *sm/sm* embryo (No. 1358; C.R.L. 4·4 mm.), 10½ days old. Dorso-ventral sections; × 335.

Figs. 3 and 4. The AER of the right hind limbs of a normal (No. 1344; C.R.L. 4·9 mm.) and of an *sm/sm* embryo (No. 1347; C.R.L. 4·8 mm.), 11 days old. Dorso-ventral sections; × 335.

Fig. 5. Transverse section through the left hind limb of an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; 12 days old) showing hyperplasia of the epidermis both on the dorsal and plantar surface; a trace of the AER still visible on the right. × 75.

Figs. 6 and 8. Dorso-ventral section through the right hind limb of a normal embryo (No. 1341; C.R.L. 5·1 mm.; 11 days old). Fig. 6, the whole limb at magnification × 75; Fig. 8, the boxed area at magnification × 335 to show the structure of the epidermis.

Figs. 7 and 9. Dorso-ventral section through the right hind limb of an *sm/sm* embryo (No. 1348; C.R.L. 5·1 mm.; 11 days old). Magnifications as in Figs. 6 and 8. Note hyperplasia of the epidermis in the boxed-in area.



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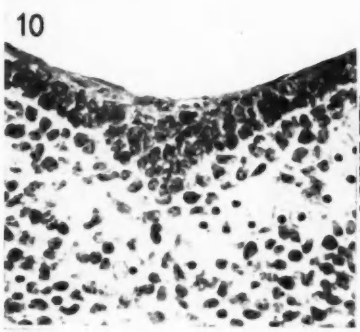
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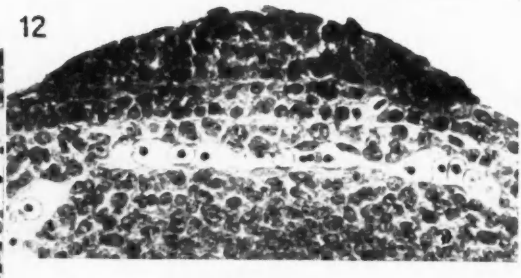
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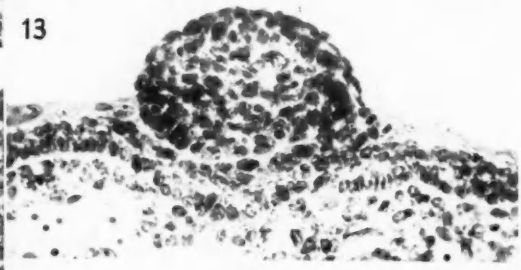
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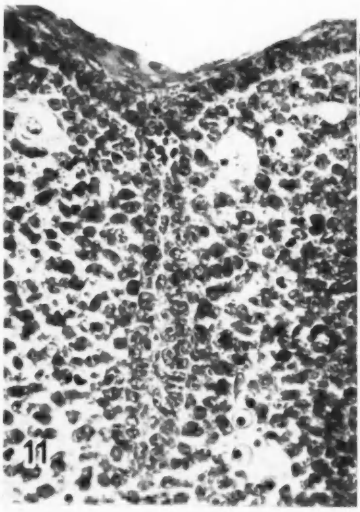
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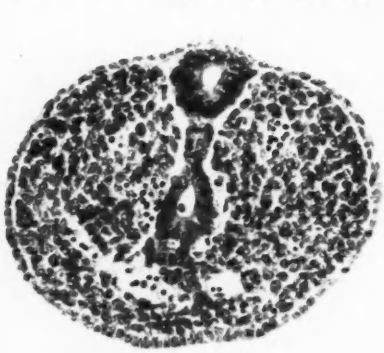
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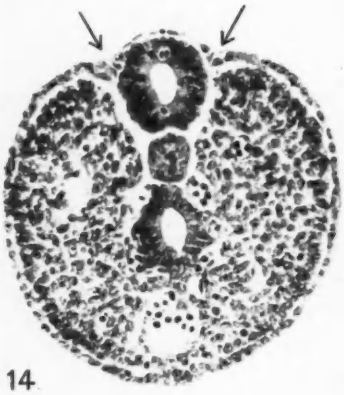
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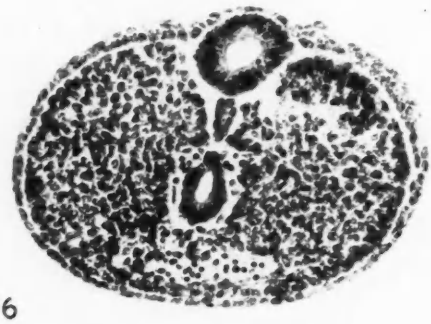
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embryos of about 4·5 mm C.R.L. (developmental age 10½ days); by the age of 11 days, the hyperplastic areas are usually fully developed. Most *sm/sm* embryos show the hyperplasia of the limb epidermis to a marked degree, but sometimes it is only slight or nearly absent. A more detailed description of the histology of this hyperplasia will have to await work with more specialized techniques.

5. *Hyperplasia of the tail epidermis*

A hyperplasia of the tail epidermis comparable to that described above for the limbs occurs in some (but not all) *sm/sm* embryos. An initial stage is shown in Fig. 14, Plate II. In this 11-day embryo, a thickening of the epidermis is present dorsally on either side of the neural tube; on top of the neural tube, the epidermis is represented by the stratum germinativum only (the lack of the periderm in this position being a normal feature of mouse embryos), and elsewhere it is also of about normal structure. The thickened area can be followed approximately from sections 32–47 (7·5 μ) as counted from the tip of the tail; the section shown is No. 39 and hence fairly distal in the tail; the ventral ectodermal ridge still being detectable in this region. At this early stage the relevant region of the tail was still straight.

A more advanced stage in a 12-day embryo is shown in Fig. 16, Plate II. The tip of the tail, for about forty-five sections, is approximately straight and cut transversely; up to section 28 (which is shown in Fig. 15, Plate II, for comparison), the epidermal structure is virtually normal; soon afterwards, a considerable thickening of the epidermis laterally and dorsally supervenes; Fig. 16 is section 42, which just grazes the last somite formed; the epidermis dorso-laterally is much thickened, and even on top of the neural tube the epidermis has become two-layered. The proximal limit of the hyperplastic area cannot be determined in this case, as on account of a sharp dorsal curvature of the tail the sections beyond No. 45 become oblique and

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PLATE II

Fig. 10. Palmar section through the right fore limb of an *sm/sm* embryo (No. 546; 13 days old) showing slight invagination of the AER into the underlying mesenchyme; externally, the ridge is covered by a cornified layer. × 335.

Fig. 11. Palmar section through the left fore limb of an *sm/sm* embryo (No. 544; 14 days old) with deep invagination of epidermis between the anlagen of two metacarpalia; parakeratosis of the top layer of the epidermis. × 335.

Fig. 12. Plantar section through the left hind limb of an *sm/sm* embryo (No. 547; 13 days old) with a large flat wart; from the top downwards wart, AER (2–3 cell layers) and mesoderm with marginal vein containing red blood cells. × 335.

Fig. 13. Plantar section through the right hind limb of an *sm/sm* embryo (No. 544; 14 days old) with a small wart flanked on either side by a thick cornified layer of epidermis. × 335.

Fig. 14. Transverse section through the tail of an *sm/sm* embryo (No. 1346; C.R.L. 4·7 mm.; 11 days old; the 39th section through the tail as counted from the tip). Beginning thickening of the epidermis on either side of the neural tube (indicated by arrows). × 190.

Figs. 15 and 16. Two transverse sections (Nos. 28 and 42 as counted from the tail tip) through the tail of an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; 12 days old). In Fig. 15 the epidermis is still normal, with a trace of the ventral ectodermal ridge just detectable; in Fig. 16 massive hyperplasia of the epidermis both dorsally and laterally; on the ventral circumference the ventral ectodermal ridge has disappeared. × 190.

thus unsuitable for this purpose. This is indeed the difficulty with the interpretation of many of the tails at this stage; hyperplastic areas are generally in the neighbourhood of the tail curvatures, and taken in isolation, it is often difficult or impossible to exclude the possibility that a thickening of the epidermis might be merely the result of oblique sections. However, there are enough clear cases to show that hyperplasia of the epidermis in the distal half of the tail is a genuine phenomenon in some *sm/sm* embryos though not all of them can be classified with confidence.

The tails of 10–10½-day-old embryos have not been sectioned for technical reasons. It is, however, improbable that they would have shown still earlier stages of the anomaly, if only for the fact that the tail region affected has not yet been formed at that time.

In all cases which could be clearly interpreted, the hyperplasia of the tail epidermis involved the dorsal rather than ventral aspect of the tail. If it may be assumed that hyperplastic epidermis presents some hindrance to longitudinal growth of the tail, the ventral side would be favoured in growth as compared with the dorsal, and a backwards turn or even twist might result. As the hyperplastic areas occur in the right region of the tail and indeed usually near tail curvatures, there can be little doubt that they are the mechanical cause of the tail kinks encountered later in life. The fact that they are variable and sometimes altogether absent is in agreement with the fact that more than half the syndactylous mice have quite normal tails.

A search for other hyperplastic epidermal regions has been carried out in the 12- and 13-day embryos which were sectioned completely. None have in fact been discovered. However, I am far from certain whether a more prolonged search, perhaps in material sectioned in a different plane, might not lead to the discovery of additional affected localities. It also remains possible that detailed histological studies might reveal minor anomalies in non-hyperplastic regions of the skin.

6. *Parakeratosis*

In the normal mouse embryo, the stratum corneum is formed on the sixteenth day (Hanson, 1947); as is known to every mouse embryologist, Bouin's fixative penetrates the skin readily up to the 16-day stage, but not thereafter. In *sm/sm* embryos, a premature keratinization occurs from the 13-day stage onwards. Unlike the fully mature keratin of the adult, and like some pathological keratinizations (e.g., psoriasis) in man, the keratin formed still contains some nuclei, many of them ghost-like and almost non-staining with haematoxylin. The process is thus one of parakeratosis.

Parakeratosis in *sm/sm* embryos occurs where epidermal hyperplasia has produced an excessive thickness of epithelial cells. The main locality is the apical ectodermal ridge; it is also found on hyperplastic patches of limb epidermis; whether it occurs on the tail is not known. Two typical deposits over sites of epidermal invaginations are shown in Figs. 10 and 11, Plate II; they come from 13- and 14-day stages respectively; even heavier masses are found in 15-day-old *sm/sm* embryos. Cornification occurs in patches. Very commonly it is found adjacent to the 'warts' which will be described in the next section; a clear case is shown in

Fig. 13, Plate II; similar cornified layers also flank the 'wart' in Fig. 12, Plate II, but are almost entirely outside the area included in the photograph.

The identification of keratin presents difficulties with which skin histologists are familiar. In the present case it rests on the typical position on top of the epidermis and on its histological resemblance to undoubted keratin encountered in the mouse and elsewhere; i.e. it is either structureless or shows some horizontal stratification, and it is eosinophil; most nuclei contained in it stain poorly or not at all. While these criteria make the identification very probable, more critical tests with special methods remain to be carried out.

7. '*Warts*'

From the age of 13 days onwards, sharply circumscribed roundish areas of the AER are separated from the underlying epithelium by a demarcating 'cut'. The material thus separated from the epidermis may form a fairly flat lentil-shaped mass, as in Fig. 12, Plate II, or a more roundish body which projects abruptly over the surface and then usually also indents the material beneath it, as in Fig. 13, Plate II. For want of a better word, and in spite of obvious differences, such structures may be called 'warts'. One of the differences is the fact that these bodies do not seem to cornify themselves though they are usually surrounded by horny masses, as mentioned above. Warts are present in many *sm*/*sm* embryos of 13 days or over, and a single limb may have more than one wart. The demarcated material shows dense nuclei and thus stains deeply. It may be assumed that warts will in the end be sloughed off, though the actual process has not been observed in the age range examined in this paper; no remains of them have been noticed at birth or afterwards. Obviously wart formation is one way to remove excess material from the region of the AER; however, only a very small fraction is eliminated in this fashion.

DISCUSSION

To simplify the discussion of the various effects of the *sm* gene described above, their age of onset has been summarized in Table 3. The earliest anomalies discovered

Table 3. *The age of onset of various anomalies of* sm/sm *embryos*

	Age in days					
	10	10½	11	12	13	14
Hyperplasia of AER (fore limbs)	(+)	+	+	+	−	
Hyperplasia of AER (hind limbs)	−	−	+	+	+	
Hyperplasia of limb epidermis	−	(+)	+	+	+	
Hyperplasia of tail epidermis			(+)	+	*	
Enlargement of the foot plates (fore limbs)	−	−	(+)	+	+	+
Enlargement of the foot plates (hind limbs)	−	−	−	+	+	+
Invagination of epidermis	−	−	−	+	+	+
Parakeratosis	−	−	−	−	+	+
Warts	−	−	−	−	+	+

* One out of two tails sectioned was straight and without hyperplasia, the other somewhat suspicious but not quite clear.

include the hyperplasia of the AER and of the limb epidermis which can be clearly identified at the age of 10–10½ days. Evidently, the anomaly develops when a critical stage of epidermal differentiation is reached. In the fore limbs the AER becomes hyperplastic at the age of 10 days, in the hind limbs at 11 days; the epidermis of the limbs becomes hyperplastic at 10½ days, that of the tail probably not (or not much) before 11 days. There can be little doubt that the epidermal hyperplasia is (potentially) a systemic anomaly, and that its manifestations in AER, the limb epidermis and the tail epidermis must be regarded as one and the same process. Similarly, there can be no reasonable doubt that parakeratosis and warts which first appear in the 13-day stage are consequences of the preceding epidermal hyperplasia. The *sm/sm* embryo thus suffers from a systemic disease of the skin, a situation which could scarcely have been foreseen when the adult skeletal anatomy was studied. So far as is known, this embryonic skin disease is transitory, as in the living adult *sm/sm* mouse skin and fur appear to be normal.

Turning now to the limb buds, enlargement and deformation of the foot plates is clearly present both in the fore and in the hind limbs in the 12-day stage; in the fore limbs, the very beginning of the anomaly can probably be traced back to the 11-day stage. It is immediately obvious that the deformation of the foot plates with the bending over of the margin in a palmar and plantar direction respectively is the mechanical cause for the crowding of the middle rays and thus for the ensuing syndactylism. All this happens before there are recognizable phalanges; the blastemata present at the 12-day stage probably represent no more than metacarpals and metatarsals respectively. One is used to the idea that the size and shape of our skeletal elements determines the shape of our bodies. In this case the situation is clearly reversed, as the size and shape of the phalanges is determined by that of the foot plates. There is reason to think that this is not a very exceptional situation.

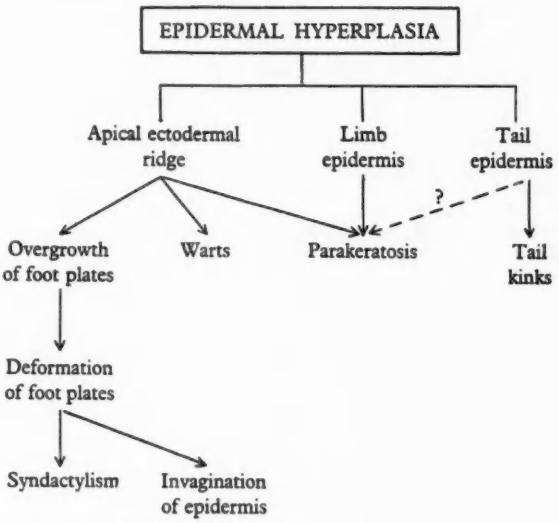
We have already discussed the origin of the epidermal invaginations; like syndactylism, these are mechanical consequences of the deformation of the foot plates.

Table 3 shows that the epidermal hyperplasia of *sm/sm* embryos can be traced back a full day beyond the earliest indications of an enlargement of the foot plates. Could the skin condition be responsible for the enlargement of the foot plates which follows it in time?

The work of Saunders (1948) in the chick embryo has shown that the AER is playing an important role in the outgrowth of the limb buds. Extirpation of the AER suppresses the formation of those parts of the limb which have not yet been formed by the apical mesodermal growth zone at the time of operation. It was also shown by Zwilling (1949) that in the wingless mutant in the chick the AER is reduced or absent. Experiments with another wingless mutant (Zwilling, 1956) with a similar regression of the AER showed that mutant AER grafted on normal mesoderm did not stimulate outgrowth; conversely, mutant mesoderm with a normal AER developed distally beyond the controls; however, after some time the AER regressed and development was not completed. From this and similar observations, Zwilling concluded that there exists a reciprocal relationship between the

mesoderm and the AER: the mesoderm grows in response to a stimulus supplied by the AER, but the AER in turn does not remain functional unless supplied with a 'maintenance factor' by the mesoderm. In the case of the wingless chicks, the fault seems to lie with the mesoderm which fails to supply the necessary maintenance factor with the result that the AER regresses and thus no longer stimulates the outgrowth of the wing bud.

There is no doubt that the AER in the chick and presumably in other organisms acts as a stimulatory organ for the growth of the underlying mesoderm. In *sm/sm* embryos, the AER is visibly hyperplastic at 10–10½ days, and about a day later enlargement of the foot plates can be demonstrated. It seems reasonable to suggest that, as reduction or absence of the AER leads to regression of the limb in wingless chickens, so hyperplasia of the AER may lead to overgrowth in the *sm/sm* mouse. The state of the AER points to a normal, but not necessarily to an increased, pro-



Text-fig. 14. The pedigree of causes of the *sm* gene.

duction of the maintenance factor: although Zwilling & Hansborough (1956) suggested that polydactylism in the chicken is due to a pre-axially more extended distribution of the maintenance factor (which acts indirectly through the AER), in other instances the AER may well be hyperactive for different reasons. In the present case, the time relations of events suggest a primary role of the AER which seems to be involved as part of a systemic tendency to epidermal hyperplasia.

If this argument is accepted, the syndactylism as well as the tail kinks of *sm/sm* mice must be regarded as secondary to an embryonic skin disease (Text-fig. 14), a relationship not hitherto described in developmental genetics.

It has sometimes been assumed that normal alleles fulfil functions in development which are similar to the phenotypic effects of the corresponding mutant genes.

For instance, if there are, say, thirty known loci which have given rise to eye colour mutations in *Drosophila*, it has been said that there must be at least thirty different steps in the elaboration of the normal eye pigments which correspond to the normal alleles of these mutants. The case under discussion demonstrates that such a conclusion may be quite misleading. The gene for *sm*, without a full embryological analysis, would presumably be regarded as a 'skeletal gene'. Yet its normal allel has obviously nothing whatever to do with the development of the skeleton.

Similarly, some writers have been tempted to deduce from an adult phenotype that such a 'skeletal gene' acts on the mesoderm. Plainly, in the present case the gene acts on the epidermis in the first instance, and its effect on skeletal structures derived from the mesoderm is secondary. However, it would be equally illegitimate to say that the *sm* gene acts on the ectoderm. It is, of course, true that the epidermis is ectodermal in origin. But so are many other structures which are not demonstrably affected by the *sm* gene. To say that *sm* affects the ectoderm would be a wanton generalization. To replace one statement by another which is manifestly less accurate is a step away from the truth.

It is widely accepted that during development genes are selectively activated at the time, and in the place, in which their services are required. The present case illustrates this principle very clearly. In normal development, keratinization of the skin happens at the 16-day stage. The hyperplastic areas of the epidermis in *sm*/*sm* embryos start to keratinize at the 13-day stage. The formation of keratin involves biochemical processes which, it must be presumed, are under the control of specific genes. In normal development, the epidermis reaches the physiological state which activates these genes at the 16-day stage, in *sm*/*sm* embryos, in certain places, some 3 days earlier. It need scarcely be pointed out that this argument would fall to the ground if the substance here tentatively identified as keratin should turn out to be something else.

So far as I know, this is the first embryological analysis of a case of inherited syndactylism. The development of various other limb anomalies which have been studied in the mouse and other animals will be discussed on a later occasion.

SUMMARY

1. The earliest manifestations of the gene for syndactylism discovered include hyperplasia of the apical ectodermal ridge of the limbs and hyperplasia of parts of the limb epidermis (10–10½-day stage). Somewhat later, a similar hyperplasia of the epidermis in the distal parts of the tail is found in some *sm*/*sm* embryos.

2. Enlargement and deformation of the foot plates in both fore and hind limbs is present in the 12-day stage; in the fore limbs it probably starts on the preceding day. The foot plates are bent over in a palmar or plantar direction respectively; the crowding together of the middle digits thus produced is the mechanical cause of syndactylism.

3. It is suggested that the enlargement of the foot plates with the ensuing deformities is due to increased stimulation of mesenchymal growth by the hyperplastic apical ectodermal ridge. On this interpretation, all the skeletal effects

(including those in the tail) are secondary to a systemic tendency to epidermal hyperplasia.

4. The hyperplastic regions of the apical ectodermal ridge and of the limb epidermis start to keratinize (parakeratosis) in the 13-day stage whereas in normal development keratinization of the skin does not start until some 3 days later.

The microscopical preparations for this paper have been made by Mrs H. Deol and by Miss H. Bartels-Walbeck and in large part by Miss Heide Schulze, who has also taken the microphotographs. Text-figs. 1 and 2 have been prepared by Mr A. J. Lee. To all of them I wish to express my appreciation. Dr W. Kocher kindly made the planimeter measurements of Table 2. The work has been supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

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Note added in proof (April 22, 1960)

Since this paper was submitted for publication, the role of the AER as a stimulatory organ for limb outgrowth has been the subject of renewed interest (Bell, E., Saunders, J. W., Jr., & Zwilling, E. Limb development in the absence of ectodermal ridge. *Nature* (*Lond.*), **184,** 1736–7, 28 Nov. 1959). It appears that in the chick embryo, limb development may proceed in the absence of the AER if the latter has been removed by methods which leave the underlying 'refractile layer of the mesoblast' intact; whether this layer is a cellular or acellular structure remains to be investigated. Pending a full publication of the facts, one may perhaps recall the mechanism of double assurance ('doppelte Sicherung') which may lead to the formation of a lens even in the absence of an eye cup. Hence though limb development may proceed in the absence of the AER, this does not necessarily mean that the AER has nothing to do with limb differentiation in the normal course of events.

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High frequency of a lethal gene (t^e) in a laboratory stock of mice

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1. INTRODUCTION

One locus affecting tail length in the house mouse is noted for its numerous mutant alleles, many of which are lethals. One allele, brachyury (T), is unique and all the others form a single group of tailless alleles jointly symbolized by t. Particular members of the group are referred to by adding superscripts as in t^0, t^{12}, t^{w11}, t^e. Homozygotes for some t alleles are viable, as also many heterozygous compounds of two different t-lethals. Homozygotes for T and for t-lethals die at various stages of embryonic development specific to each allele; Tt mice are tailless; $T+$ short-tailed; $+t$ and viable animals with two t's are normal-tailed. Although segregation from heterozygous females is quite normal, males that are heterozygous for almost any t-lethal transmit it to much more than half their offspring (Dunn, 1957; Dunn & Gluecksohn-Schoenheimer, 1950). This anomaly is clearly shown in test-matings to which the allele T is introduced so that the two types of spermatozoa leave distinguishable offspring. For example, when Tt fathers are paired with $++$ mothers, most offspring receive the father's t gene and are $+t$, while few are $T+$. Similarly, when spermatozoa of $+t$ fathers fertilize T eggs from $T+$ and Tt mothers, there are far more Tt than $T+$ offspring (products of the other eggs are disregarded here, being either indistinguishable from normal or inviable). Segregations in such test-matings have been used as direct measures of the frequency of t in effective spermatozoa. The excessive transmission of t-lethals by effective spermatozoa confers such powerful advantages on the genes that, in spite of their lethality, they often occur at high frequencies in natural populations (Dunn, Beasley & Tinker, 1958). Theoretically, there is no limit to the proportion of phenotypically normal individuals that could carry a particular lethal, and if t was transmitted by all effective spermatozoa from $+t$ males a stable situation would rapidly be reached where every living individual was heterozygous for t (Bruck, 1957). However, the proportion of $+t$ mice does not appear to be entirely determined by the frequency of t-bearing spermatozoa. Thus Dunn *et al.* (1958) reported fewer heterozygotes for t-lethals in fourteen feral populations than would be expected from the average frequency of t-lethals for effective spermatozoa in test-matings. This discrepancy was aggravated by finding that $+t$ males were more viable in the laboratory than homozygous normals.

It was precisely to avoid the uncertain validity of parameters estimated for feral

mice under laboratory conditions that the present work was undertaken with a laboratory stock. Yet even here, as will be seen later, the transmission of t by effective spermatozoa inadequately accounted for the $+t$ mice that this time were too numerous. Besides reporting this situation, the present paper examines the assumptions made in comparing actual and expected frequencies of $+t$ mice, namely: the laboratory stock segregated for only one t allele and this was fully lethal; stock $+t$ and $++$ mice were equally viable and fertile; in the test-matings used for assessing the frequency of t and $+$ spermatozoa, Tt and $T+$ offspring were equally viable, and the phenotypes of these offspring fully penetrant. As these assumptions are found valid it is concluded that the frequencies of t-bearing spermatozoa assessed for test-matings cannot legitimately be applied to stock matings. It is supposed that the segregation of t-lethals in effective spermatozoa is not purely a paternal characteristic but is also a property of the mother or her eggs.

2. MATERIAL

The present investigation makes use of two kinds of animals—'stock' and 'testers'. Stock animals are from the laboratory stock which segregated naturally for the t-lethal of this investigation; testers are hybrid animals made heterozygous for the allelomorph T.

The precise origin of the t-lethal is not known as the stock was based on a number of inbred lines and heterozygous populations. Breeding has since continued for thirty generations within a closed population, and for the last seventeen generations two sub-populations have been maintained. In the tables these are referred to as lines A and B. The investigations were made on the sixteenth and seventeenth generations following the separation. The lethal gene, which was first found by Dr A. W. H. Braden, occurs in both lines and has been named tailless-Edinburgh (t^e) (Bateman, 1957). To facilitate analysis of the genetic situation, stock animals were mated individually to testers of the constitution $T+$ or Tt^e. These were obtained from earlier crosses between stock animals (which supplied $+$ and t^e) and an unrelated strain that contributed T. A list of the genes and genotypes relevant to the investigation is given in Table 1.

Table 1. *List of genes and viable genotypes at the* t*-locus*

Symbol	Full name
	Genes
t^e	tailless-Edinburgh (lethal)
$+$	normal
T	brachyury (lethal)
	Viable genotypes
$++$	normal-tailed, homozygous normal
$+t^e$	normal-tailed, carrier
$T+$	short-tailed
Tt^e	tailless

3. METHODS

Various experimental methods were employed details of which are perhaps best deferred. Here, it is intended to describe only the more general methods. Usually, mice from the laboratory stock, all of which looked normal, were mated to tester animals heterozygous for brachyury (T). Homozygotes would have been better, but the brachyury gene is unfortunately itself a lethal. The gametic unions of t^e with T and of $+$ with T resulted in tailless and short-tailed individuals. These unions were useful for distinguishing carriers from homozygous normals, and for estimating gametic ratios of $t^e : +$. In both cases, information was gained only from conjugations involving the testers' T gametes, and in this regard, Tt^e and $T+$ females were equally suitable. Tt^e males were not used in the tests because of the rarity with which they produced offspring by their T-bearing spermatozoa. No useful information could be gained from t^e and $+$ gametes uniting with similar gametes from the testers, as the surviving foetuses were all normal-tailed. These progeny of the test-matings were therefore disregarded. The progeny were classified on their day of birth when the three phenotypes could be distinguished without error.

4. RESULTS

Lethality of homozygotes

Not all genes that form tailless zygotes with the brachyury gene give lethal homozygotes, and the lethality of new tailless genes cannot be assumed. The viable combinations of tailless genes (either homozygotes for a viable, or heterozygotes for two different lethals) are normal-tailed. Homozygotes for brachyury die in early gestation. The usual test of the lethality of new genes is to mate tailless mice together, $Tt \times Tt$. If the t gene is a lethal, a balanced lethal stock is formed where TT and tt zygotes die *in utero* and only Tt heterozygotes survive; all the offspring are then tailless. But if the t gene is a viable, tt offspring survive and are recognized by their normal tails. Eight $Tt^e \times Tt^e$ matings were made between testers: all their sixty-six progeny were tailless, showing t^e to be a lethal gene.

It was felt necessary to ascertain that t^e was also lethal in the laboratory stock itself, and to ensure that no other tailless allele (t^x) segregated. For the excess of carriers discovered when tt mice were expected to die (see Interpretation) might well disappear if some tt mice (whether $t^e t^e$, $t^e t^x$ or $t^x t^x$) survived. Survival of tt males would in no way affect the situation because, being invariably sterile, their genotypes could not be tested nor could they contribute genes to subsequent generations. However, tt females are fertile, and they affect the results in two ways. Their number should be deducted from the apparent number of $+t$ mice, and the expectation of $+t$ should be increased on account of the additional number of t eggs tt females provide. The excess of carriers would disappear if about one-quarter of supposed $+t$ females were fertile tt females. Accordingly, a test of heterozygosity was made as follows. Stock females that had borne no short-tailed offspring from their test-matings, but were known to carry one t gene because they had produced tailless offspring, were re-mated to $T+$ tester males. Whereas $+t$ females could

have both tailless and short-tailed offspring, *tt* females would have no short-tailed offspring. Eventually, out of one hundred females tested, ninety-nine proved to be $+t$; i.e. they had both tailless and short-tailed offspring. The remaining female provided no information on heterozygosity as she bore only a single tailless offspring. Tailless-Edinburgh is therefore established as the only *t* allele segregating in the stock and as a fully lethal gene.

Survival of normals and carriers

The genotypes were tested for forty-nine progeny from stock matings that, having been made between $+t^e$ ♀♀ × + + ♂♂, were expected to yield carriers and homozygous normals in equal numbers. Actually, 23 $+t^e$'s and 26 + +'s were observed, i.e. $0{\cdot}47 \pm 0{\cdot}07$ were $+t^e$'s. Most likely animals of these genotypes were equally viable up to the time of the test, and long after they would normally have weaned the next generation and have been discarded. Although the numbers were small, there was clearly no repeat of the situation reported by Dunn *et al.* (1958) for similar progenies of feral extraction for which the ratio was 35 : 18.

Fertility of carriers and normals

It was routine practice in managing the laboratory stock to leave the males and females together for 16 days after pairing, and to discard as 'failed' those parents that did not conceive within this period. In the second of the generations studied here both failed and successful parents were re-mated to $T+$ and Tt^e testers and eventually all but nine of the intended parents were tested. The incidence of carriers and normals is shown for each class in Table 2. There was no evidence that carriers and homozygous normals differed in fertility.

Table 2. *Indicence of carriers and homozygous normals among failed and successful parents.* (*Observations on males weighted by number of matings*)

Sex	Stock mating	Tested: Total	Tested: Carriers	Tested: Normals	Not tested
Dams	Failed	33	29	4	9
	Successful	91	71	20	0
	Total	124	100	24	
Sires	Failed	39	33	6	0
	Successful	94	71	23	0
	Total	133	104	29	
Sexes combined	Failed	72	62	10	9
	Successful	185	142	43	0
	Total	257	204	53	

Heterogeneity tests:

Source	D.F.	χ^2	*P*
Within sexes	2	2·83	0·25
Sexes combined	1	2·77	0·10
Dam-sire interaction	1	0·06	0·80

Efficacy of the zygotic $Tt^e : T+$ *ratio as a measure of the gametic* $t^e : +$ *ratio*

The ratio of $t^e : +$ gametes for stock males was to be measured by the zygotic ratio of $Tt^e : T+$ in their test litters, and it was therefore necessary to find whether Tt^e and $T+$ embryos were equally viable. Information from the males' own test litters was scanty. The question was whether similar numbers of T eggs from tester females were represented by the birth of predominantly Tt^e litters sired by $+t^e$ males as in the entirely $T+$ litters (always disregarding normal-tailed offspring) of $++$ males. The comparison was confined to first litters. In view of the small number of $++$ males tested it was expedient to pool the information from successive generations, from A and B lines, and from Tt^e and $T+$ tester females. This procedure appeared valid as group means respecting $+t^e$ males were quite unform. A mean number of $3{\cdot}65 \pm 0{\cdot}22$ tailless and short-tailed offspring were born to $+t^e$ males, and $3{\cdot}50 \pm 0{\cdot}39$ short-tailed offspring to $++$ males. This evidence on differential mortality of Tt^e and $T+$ embryos is quite uncritical.

It was possible with the test litters born to a much larger number of $+t^e$ females to arrive at critical, if indirect, evidence on this matter. Because $+t^e$ females segregate normally it would be reasonable to expect their test litters to produce equal numbers of Tt^e and $T+$ offspring. Actually, a slight bias favouring Tt^e embryos was expected in the overall figures for the following reason. The litters were originally required to determine the genotypes of the tested parents, so no further litters were raised after the first diagnostic Tt^e young appeared. In consequence, test litters of $+t^e$ females that had only one young were 100% Tt^e, instead of the $50 : 50$ $Tt^e : T+$ expected in an unbiased sample. Larger litters would be subject to bias of a lesser order. The bias was excluded by restricting the data to litters that contained both Tt^e and $T+$ offspring. The numbers of tailless and short-tailed offspring in this material were $94 : 97$ for one generation and $162 : 157$ for the other. In neither generation was there any evidence of differential viability of Tt^e and $T+$ embryos. Unless other mothers react violently to these embryos, it is to be concluded that their peculiar frequencies in the test litters of $+t^e$ stock males mirror the underlying ratio of $t^e : +$ genes in the spermatozoa which were successful in fertilization.

Proportion of carriers

Individuals from two successive generations of the stock A and B lines were tested with $T+$ and Tt^e mates. Each individual was either diagnosed as a carrier by the appearance of tailless progeny, or was regarded as a homozygous normal if no tailless but four or more short-tailed progeny appeared. The average number of short-tailed progeny for individuals in this latter class was actually 6·5 and the probability of a carrier escaping recognition was thus usually considerably less than one-sixteenth. The frequency of carriers may therefore be slightly underestimated, but certainly not overestimated. Table 3 gives the numbers of carriers and normals for each of the four subdivisions of the stock. Since the results were homogeneous, although the two lines had been maintained separately for many generations, the proportion of carriers is understood to have reached a stable level in both lines. The figures show

that 80% of individuals were carriers, and this is taken to be their equilibrium frequency.

Table 3. *Numbers of carriers and homozygous normals in four subdivisions of the tested male and female breeding stock*

Line	Generation	Total tested	Carriers	Normals	Expectation
A	1st	50	36	14	40:10
	2nd	85	67	18	68:17
B	1st	43	38	5	34: 9
	2nd	86	70	16	69:17
	Total	264	211	53	211:53

Heterogeneity tests:

Source	D.F.	χ^2	P
Lines	1	2·27	0·14
Generations	1	0·01	0·91
Interaction	1	1·77	0·18
Overall	3	4·05	0·25

Overall frequency of carriers ($+t^e$) = 211/264 = 80%.

Percentage t^e *in effective spermatozoa from all stock males*

The proportion of t^e and + spermatozoa uniting with T eggs was determined for all stock males (carriers and normals) from the numbers of Tt^e and $T+$ progeny in their first litters from matings to Tt^e and $T+$ tester females (Table 4). The four subdivisions of the stock, provided by two lines in two generations, are shown to

Table 4. *Segregation of* Tt^e *and* T+ *progeny in the first test litters of stock males mated to* Tt^e *or* T+ *tester females. (Normal-tailed young excluded)*

		Progeny			
Line	Generation	Total	Tt^e	$T+$	Expectation
A	1st	87	60	27	62:25
	2nd	83	54	29	59:24
B	1st	72	54	18	51:21
	2nd	92	69	23	65:27
	Total	334	237	97	237:97

Heterogeneity tests:

Source	D.F.	χ^2	P
Lines	1	2·55	0·11
Generations	1	0·08	0·76
Interaction	1	0·50	0·48
Overall	3	3·13	0·40

Overall frequency with which t^e was transmitted by the effective spermatozoa of all stock males = 237/334 = 71%.

have given homogeneous results and lead to the combined estimate that 71% of the entire sperm population fertilizing *T* eggs transmitted t^e. It would normally be presumed that t^e was transmitted in the same frequency by spermatozoa fertilizing other eggs.

Percentage of t^e *spermatozoa from* $+t^e$ *stock males*

The number of t^e and + genes among the effective spermatozoa of carriers only was estimated as the incidence of Tt^e and $T+$ progeny in the first test litters of stock males thereby (or subsequently) proven to be carriers. It can be seen from Table 5 that the four independent estimates of the ratio of t^e-bearing to +-bearing spermatozoa fertilizing *T* eggs are homogeneous, and that t^e entered 87% of those spermatozoa from carriers. Dunn's school refer to this ratio as the male segregation ratio advantage.

Table 5. *Segregation of* Tt^e *and* $T+$ *progeny in the first test litters of known carrier males in matings with* Tt^e *or* $T+$ *tester females.* (*Normal-tailed progeny excluded*)

		Progeny			
Line	Generation	Total	Tt^e	$T+$	Expectation
A	1st	71	60	11	62: 9
	2nd	61	54	7	53: 8
B	1st	61	54	7	53: 8
	2nd	78	69	9	68:10
	Total	271	237	34	237:34

Heterogeneity tests

Source	D.F.	χ^2	*P*
Lines	1	0·28	0·60
Generations	1	0·28	0·60
Interaction	1	0·35	0·55
Overall	3	0·91	0·80

Overall frequency of t^e in effective spermatozoa from stock $+t^e$ males = 237/271 = 87%.

The offspring of carrier males $(+t^e)$ *from stock matings with normal females* $(++)$

The genotypes were obtained for twenty-nine offspring from stock matings between normal females and carrier males and all proved to be carriers. If the estimate (based on conjugations with *T* eggs) that t^e entered 87% spermatozoa from $+t^e$ males applied equally to matings with normal stock females, some 3·2 normal progeny would be expected among the twenty-nine tested. The chance that no homozygous normals were found would be $0{\cdot}87^{29}$, or 0·02. It appears unlikely that the frequency of t^e spermatozoa was the same in test and stock matings.

5. INTERPRETATION

The proportion of carriers expected in any generation depends on the frequencies of t^e genes for uniting gametes, and may be found as follows. Let *p* be the proportion

of carriers among the parents and P be the proportion of offspring of carrier males that receive t^e from their fathers. Then $\frac{1}{2}p$ eggs contributing to the next generation carry t^e and $1-\frac{1}{2}p$ carry $+$; whereas pP spermatozoa carry t^e and $1-pP$ carry $+$. Under random and non-assortative mating, $\frac{1}{2}p.pP$ gametic unions give lethal, $t^e t^e$, zygotes and only the remaining $1-\frac{1}{2}p.pP$ survive. The number of carriers

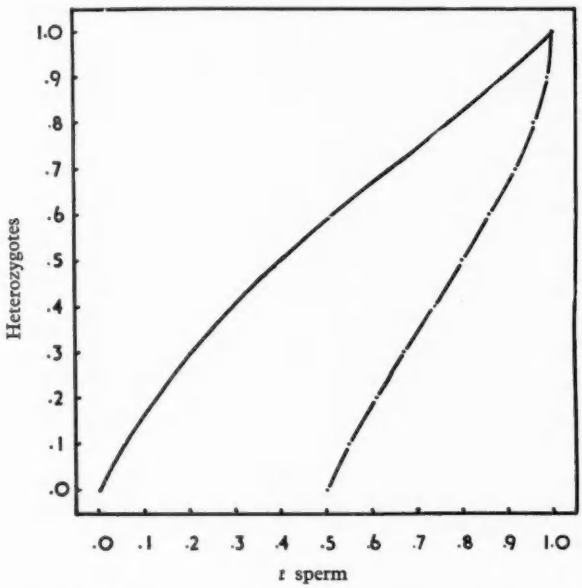

Fig. 1. Computed relationships at equilibrium between the incidence of heterozygotes and (*a*) the frequency of *t*-lethal sperm from the whole male population (continuous line) and (*b*) the segregation ratio of *t*-lethals in the sperm of heterozygous males (broken line).

among these is $\frac{1}{2}p.(1-pP)$ formed when t^e eggs are fertilized by $+$ spermatozoa, together with $(1-\frac{1}{2}p).pP$ from $+$ eggs fertilized by t^e spermatozoa. Thus, the proportion of carriers among the surviving offspring will be

$$p_1 = \frac{\frac{1}{2}p.(1-pP)+(1-\frac{1}{2}p).pP}{1-\frac{1}{2}p.pP}.$$

Eventually, p settles to an equilibrium value so that $p_1 = p$, reducing the above equation to $p = 1-\left(\frac{1}{P}-1\right)^{\frac{1}{2}}$. P then determines p. This relation was previously derived by Bruck (1957). (An incorrect relation provided by Prout (1953) arose through omitting the restriction that eggs could not be fertilized by eggs, nor spermatozoa by spermatozoa.) Where p is known, expected equilibria for pP and P can be found from the equations

$$pP = \frac{p}{1+(1-p)^2} \quad \text{and} \quad P = \frac{1}{1+(1-p)^2}.$$

The continuous curve in Fig. 1 shows the relation of p to pP, and the broken curve relates p to P. It should be mentioned that about fifteen families per line constituted each generation of the stock. Inbreeding was minimized by pairing least-related individuals together. These departures from the model are not believed seriously to affect its applicability to the stock.

Observed values for the stock were

p = proportion of carriers = 0·80 (Table 3).
pP = proportion of offspring receiving t^e from their fathers = 0·71 (Table 4).
P = proportion of offspring of $+t^e$ males receiving t^e from their fathers = 0·87 (Table 5).

The evidence of Tables 3, 4 and 5 shows these figures represent equilibrium values as they are stable between successive generations and between lines with a distant common origin. The expected value of pP is $p \times P = 0{\cdot}70$; the agreement is so close that this compound parameter can be dropped from further consideration. However, P and p do not agree. Thus, the proportion of carriers expected from $P = 0{\cdot}87$ is $p = 0{\cdot}62$ (not 0·80), and conversely, $p = 0{\cdot}80$ gives the expected proportion of offspring from $+t^e$ males receiving their fathers' t^e genes as $P = 0{\cdot}96$ (not 0·87). It was nevertheless conceivable that the observations might yet be compatible with an intermediate value of P (and p). Dr E. C. R. Reeve, in an appendix to this paper, has developed the maximum likelihood solution appropriate to finding a single P that best fits both observations. His solution is $P = 0{\cdot}928$; $p = 0{\cdot}721$. Comparison of the data with these expectations (Table 6) yields $\chi^2_{(1)} = 19{\cdot}6$, Probability $\ll 0{\cdot}001$.

Table 6. *Contingency of observed* P *with observed* p *assessed against the maximum likelihood expectation that population* P = 0·928 *and the equivalent* p = 0·721

	Spermatozoa		
Estimate	Total	t^e	+
P	271	237 (251·49)	34 (19·51)
	Individuals		
	Total	$+t^e$	++
p	264	211 (190·34)	53 (73·66)
χ^2	(1 d.f.)	19·6	$P \ll 0{\cdot}001$

In fact, carriers are far more numerous than could reasonably be expected. What factor has been overlooked?

A clue to the situation lies in the observation in stock matings that twenty-nine carriers and no normals were found among the progeny of carrier males mated to normal females. Where P is the proportion of t^e spermatozoa from carriers, the probability that no normal spermatozoa leave offspring is P raised to the twenty-ninth power. The indirect estimate $P = 0{\cdot}96$ (based on the frequency of carriers in

the stock as a whole) leads to a greater likelihood of finding only carriers in this particular section of the stock (Probability = 0·31) than the direct estimate $P = 0{\cdot}87$ obtained from the fertilization of brachyury eggs (Probability = 0·02).

It is inferred that segregations measured with the assistance of the brachyury gene are not strictly applicable to situations from which this gene is absent. If this conclusion is general, inconsistencies reported by Dunn, Beasley and Tinker may be similarly resolved. It is possible that the frequencies of the two classes of effective spermatozoa from *t* heterozygotes are specific to the genotypes of the eggs available for fertilization. Thus, if a female is heterozygous, say $T+$, the brachyury and normal eggs would return different ratios for the genes transmitted by the spermatozoa.

SUMMARY

A long-established laboratory stock was found to contain many individuals that were heterozygous for a lethal gene, called tailless-Edinburgh (t^e). Heterozygotes are indistinguishable from normals except by breeding tests with special tester stocks supplying brachyury (T) gametes, when tailless (Tt^e) progeny distinguish carrier parents from normal parents that produce only short-tailed ($T+$) progeny. When males are mated to tester females providing brachyury eggs, the ratio of $Tt^e : T+$ progeny reflects the ratio of $t^e : +$ spermatozoa. The proportion of t^e spermatozoa measured in this way led to the expectation that 62% of individuals in the original stock would be carriers, whereas 80% was found. Independent evidence is presented for $+t^e$ males that the incidence of t^e in their effective spermatozoa was higher when normal eggs were fertilized in matings within the original stock than when brachyury eggs were fertilized in outcross matings to the tester stock. These observations suggest that the proportion of t^e spermatozoa partaking in fertilization was modified by the genotypes of the females or of their eggs.

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NOTE ADDED IN PROOF

Recent information, not affecting the conclusions of the present paper, renders the account of the T-locus as given in the Introduction incorrect in two details.

Brachyury (T) is no longer unique: a second dominant allele named T-Harwell (T^h) has been described, only differing from the former in the age at which homozygotes die and in recombination frequencies when associated with some genes (Lyon, 1959).

And there are now three categories of mutant—(i) brachyury (T) alleles that shorten the tails of $T+$ heterozygotes; (ii) tailless (t) alleles that enhance the heterozygous effects of T and T^h; and the novelty (iii) a *suppressor* allele (t^{h7}, provisional symbol) which inhibits the heterozygous expression of T. Quite unlike Tt mice, Tt^{h7} mice are never tailless, have short tails only rarely, and usually have tails of normal length (Lyon, 1960).

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APPENDIX: ARE THE VALUES OF p AND P CONSISTENT WITH EQUILIBRIUM?

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We wish to test whether the observed values of p and P are consistent with the hypothesis that the population is in equilibrium such that

$$p = 1-\left(\frac{1}{P}-1\right)^{\frac{1}{2}} = 1-(Q/P)^{\frac{1}{2}}, \tag{1}$$

where $Q = 1-P$.

For this purpose, we shall calculate the maximum likelihood estimates of the two parameters, subject to the condition of equation (1), and test their agreement with the observed values of Table 3 (for p) and Table 5 (for P). The estimate of pP from Table 4 will not be used, since this largely duplicates the data of Table 5.

The likelihood equation from Tables 3 and 5 is

$$\log L = 211\log p + 53\log(1-p) + 237\log P + 34\log(1-P) \tag{2}$$

which contains only one independent variate, since p and P are related by equation (1). It may be maximized for P, using the relation derived from (1),

$$\frac{dp}{dP} = 1/2P\sqrt{(PQ)},$$

and, on putting $d(\log L)/dP = 0$, we obtain

$$\frac{211}{2P(\sqrt{(PQ)}-Q)} - \frac{53}{2PQ} + \frac{237}{P} - \frac{34}{1-P} = 0.$$

This leads to the cubic equation in Q,

$$Q^3 - 1{\cdot}335{,}793Q^2 + 0{\cdot}435{,}773Q - 0{\cdot}024{,}920 = 0,$$

of which the roots are 0·86562, 0·39780 and 0·07236. The last of these yields our estimate of P:

$$P = 0{\cdot}9276,$$

whence

$$p = 0{\cdot}7207.$$

Using these two estimates in Tables 5 and 3, respectively, we can calculate the contribution to χ^2 of the deviation of observed from expected in each table, and the sum of the two values gives a χ^2 with one degree of freedom, since one has been lost in using equation (1). The result is shown below:

	Observed—Expected	Contribution to χ^2
Table 3 (p)	14·4	11·4
Table 5 (P)	20·7	8·1
	χ^2 for one D.F.	19·5

The deviation from expectation is highly significant, the observed P being too low and the observed p too high.

It should be noted that this test is not exact, and will tend to overestimate the significance of the deviations, since it does not take account of the fact that the samples were obtained from a population of finite size. Given equation (1) above, p will have a sampling variance about its equilibrium value for a particular P, and this variance will depend on the population size and will only be zero when the latter is infinite. Equation (1) should strictly be replaced by a probability distribution of p, given P and the size of the population. In the present case, however, the deviations of p and P from their expected values are much too great to be explained by this cause of bias, and it was not considered necessary to apply a more exact test.

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Selective fertilization at the T-locus of the mouse

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1. INTRODUCTION

The route to fertilization is through gametogenesis, transport of gametes to the site of fertilization, and conjugation. Fertilization is expected to lead to equal recovery in the newly-formed zygotes of alleles that were heterozygous in the parents. The term selective fertilization has been applied to many instances when the recovery was unequal, but it is perhaps not generally realized from what devious sources and with what individually indentifiable genetic consequences the inequality may arise. Accordingly, an analysis of selective fertilization in all its possible forms is sketched in the preliminary part of this paper.

However, the main reason for the paper is to describe a new form of selective fertilization from the mouse. It will be shown that spermatozoa transmitting the t^e allele of the T-locus did not fertilize equal numbers of alternative eggs from heterozygous females. The T-locus, which gets its symbol from effects on tail length, has been extensively reviewed by Grüneberg (1952) and by Dunn & Gluecksohn-Waelsch (1953). It has many alleles, but only the three whose zygotes are set out in Table 1 need be considered for present purposes. The alleles T, $+$, and t^e are

Table 1. *List of genotypes at the T-locus*

Symbol	Full name
$+\ +$	Normal-tailed, normal homozygote
$+\ t^e$	Normal-tailed, carrier
$t^e\ t^e$	Tailless-Edinburgh homozygote (dies *in utero*)
$T\ +$	Short-tailed
$T\ t^e$	Tailless
$T\ T$	Brachyury homozygote (dies *in utero*)

brachyury, normal and tailless-Edinburgh, respectively. Both t^e and T are lethal to homozygotes, death occurring *in utero*. Other features of t^e are that it is one of many t genes (mostly lethals) which are all without phenotypic effects in heterozygous compounds with the normal gene, but which give tailless zygotes with brachury; and, like most other t genes, t^e is transmitted by heterozygous males to the great majority of their effective spermatozoa. Dunn (1953) has anticipated that t genes with high degrees of transmission in the spermatozoa would be widespread among wild mice, and this is indeed so. The exact mathematical relation between the frequency of heterozygotes and the frequency with which heterozygous

males transmit t to their spermatozoa has been worked out by Bruck (1957), but neither natural populations (Dunn, Beasley & Tinker, 1958) nor the only laboratory population which has been studied (Bateman, 1960) agrees with Bruck's predictions. As yet, no satisfactory explanation of these discrepancies has been found, but the possibility that eggs and spermatozoa might have conjugated selectively had not been investigated. However, it has been shown in the laboratory population (Bateman, 1960) that in matings with $+t^e$ males, fertilization of T eggs was less frequently due to t^e spermatozoa (87%) than were fertilizations of $+$ and t^e eggs (96–100%). As the comparison cut across females of different genotypes it was not possible to determine from the data whether any part of the difference resided with the eggs themselves. However, new evidence will be presented here that the alternative genotypes of eggs found in heterozygous females do modify the advantages in fertilization that t^e spermatozoa have over those carrying the $+$ or T alleles.

2. TYPES OF FERTILIZATION

Selective fertilization may be defined as unequal sampling in newly formed zygotes of genes that were heterozygous in parents. The selection may occur during gametogenesis, transport or conjugation. Figure 1 depicts the various consequences in terms of mothers heterozygous for alleles a and b, and fathers heterozygous for c and d. The ratio of the four zygotic classes ac, ad, bc, bd is represented by the relative areas of the respective cells whose dimensions are determined by the proportions in which parental genes are combined by the uniting gametes. Bold divisions demarcate classes that are expected to be of equal size.

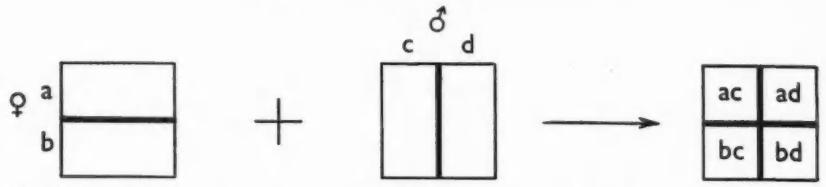
Normal fertilization (Fig. 1A)

In normal matings between heterozygotes, equal numbers of maternal alleles (represented by two equal rectangles one above the other, Fig. 1A i) combine with equal numbers of paternal alleles (two equal rectangles side by side, Fig. 1A ii) to give four zygotic classes of equal size (Fig. 1A iii). From this basic Mendelian ratio of 1 : 1 : 1 : 1 the more familiar 3 : 1, 1 : 2 : 1 and 1 : 1 ratios are derived to accord with the number of alleles and their dominance relations. Mendelian ratios may be disturbed *after* the formation of zygotes by phenotypic overlapping and different zygotic viabilities. As the aberrant segregations of most 'bad' genes are accommodated in these terms, proof of selective fertilization can be accepted only after these sources of non-Mendelian segregation are rigorously excluded.

Selective gametogenesis (Fig. 1B)

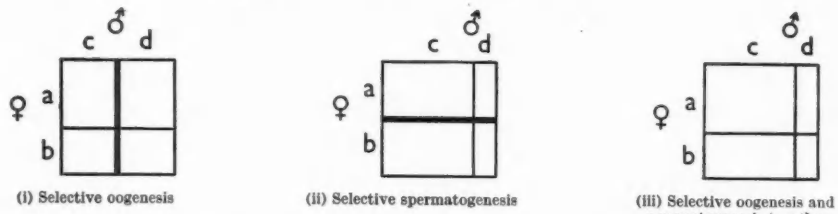
If certain alleles tend to be eliminated from the germ line by the meiotic process, this is selective gametogenesis. The resulting inequality of parental alleles in the offspring is of course uninfluenced by conditions of mating. Thus the predominantly male or female progenies of mice in lines bred for high or low blood pH are almost certainly the product of selective spermatogenesis. The father's origin determines the predominant sex of the offspring in outcrosses even when insemination is

(A) NORMAL FERTILIZATION



(i) Female pronuclei carrying equal numbers of maternal alleles — and — (ii) Male pronuclei carrying equal numbers of paternal alleles — conjugate at random to give — (iii) Four equal zygotic classes

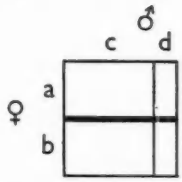
(B) SELECTIVE GAMETOGENESIS



(i) Selective oogenesis (ii) Selective spermatogenesis (iii) Selective oogenesis and spermatogenesis together

a : b and c : d are invariant ratios, being characters of the parents.

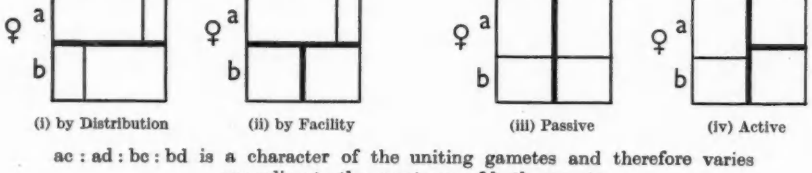
(C) SELECTIVE SPERM TRANSPORT



c : d is a character of the male or female genital tracts and may vary with the mother and with appropriate techniques of semen collection and insemination.

(D) SELECTIVE CONJUGATION

SELECTIVE PENETRATION SELECTIVE RETENTION



(i) by Distribution (ii) by Facility (iii) Passive (iv) Active

ac : ad : bc : bd is a character of the uniting gametes and therefore varies according to the genotypes of both parents.

Fig. 1. Causes of selective fertilization, and results expected.

artificial and semen is taken from the vas deferens (Weir, 1958). Likewise in male *Drosophila* that are heterozygous for segregation distorter. Close synapsis in spermatogenesis of normal and SD homologues is thought to break the normal chromosome with the result that normal, viable spermatozoa are rarely produced and SD is recovered in 90% of the offspring (Sandler, Hiraizumi & Sandler, 1959). Fully authenticated cases of selective gametogenesis are the expulsion of larger chromosomes of unequal pairs in polar bodies (Sandler & Novitski, 1957); replication of X chromosomes with compensatory loss of Y's, as cited by Wallace (1948) for sex-ratio strains of *Drosophila*; and gene conversion (Lindegren & Lindegren, 1956).

Selective transport (Fig. 1C)

Selective transport of gametes is indicated when changing the conditions of mating or changing the mate affects gene frequencies in the offspring. Vertebrate eggs are not open to such selection because gametogenesis and conjugation overlap. On the other hand, vertebrate spermatozoa may be selected during transport in both the father's and mother's genital tracts. Thus, in contradistinction to selective spermatogenesis (Fig. 1B ii), the anomalies of selective sperm transport can vary with the mother, and according to such conditions as (i) whether matings are natural or artificial, (ii) where the spermatozoa are taken from and where inseminated, (iii) the time spermatozoa spend in the tracts. Such anomalies have been described for mice (Braden, 1958), where the timing of coitus in respect of oestrus alters the relative fertilizing capacities of spermatozoa carrying *t* genes and their alleles. According to Braden & Gluecksohn-Waelsch (1958) the junction of the uterus with the Fallopian tubes is a site of stringent sperm selection. In many monoecious plants self-fertilization is inhibited by the slow growth of pollen tubes transporting genes that are the same as ones present in the style.

Selective conjugation (Fig. 1D)

In matings between heterozygotes it may be possible to show that nuclear conjugations are not randomly disposed among gametes which reach the site of fertilization. Selective conjugation reflects special associations among gametic nuclei carrying particular alleles. Eggs of non-vertebrate organisms undergo both meiotic divisions before becoming available for spermatozoa to penetrate. The eggs of these organisms are therefore fully differentiated at penetration in regard to the genes they carry, so that in these organisms selective conjugation of nuclei is simply the consequence of 'selective penetration' among gametes (Fig. 1D i–ii).

The significance of this point is clarified when it is remembered that vertebrate eggs are penetrated after completing only their first meiotic division (Beatty, 1957). Although this division is fully reductional in the sense of halving the number of bivalent chromosomes and of segregating alternative alleles in non-crossover segments, heterozygosity in crossover segments remains until sister chromatids separate at the second division (White, 1945). Non-crossover segments are likely to include loci that are close to the centromeres; and crossover segments the more distal loci. Vertebrate eggs can therefore be categorized in regard to a locus for

which conjugation is selective according to whether this locus segregates at the first or second meiotic division. Eggs of the first category are distinguished before penetration by the one allele each carries, and like non-vertebrate eggs are therefore accessible to selective penetration. This is clearly ruled out if the locus does not segregate till the second meiotic division, because in this case the eggs had uniformly carried both alleles when they were penetrated; but when such eggs divide again, should the spermatozoa retain selected alleles and eliminate the alternatives in the second polar bodies, selective conjugation of a different type will be effected. This type is distinguished as 'selective retention' (Fig. 1D iii–iv). The distinction is significant genetically, as the mode of conjugation determines which parent's alleles will be unequally inherited. Assuming all eggs are fertilized, the mechanics of normal meiosis in selective penetration ensures equal recovery of the mother's genes. However, the superior ability to fertilize certain eggs which is given to spermatozoa carrying one of the father's alleles biases the recovery of these. (As described later in this paper, these features appear in fertilizations involving t^e spermatozoa and mark the anomalies there as being due to selective penetration.) The contrary features obtain for selective retention. Here, the uniformity of penetrated eggs ensures equal entry of the two classes of spermatozoa into the zygotes, while spermatic interference in the egg's second meiotic division results in unequal participation of the mother's genes. The existence of selective retention has not yet been established, although its prerequisites are perhaps not very different from selective oogenesis.

It is of value to subdivide both forms of selective conjugation. For example, selective penetration may be the result of inequalities in the distribution of spermatozoa about the eggs, or of the unequal facility with which individual spermatozoa penetrate them. In the mouse, where eggs are tightly clustered at the site of fertilization (Braden, private communication) and few spermatozoa reach them (Braden, 1957), it could happen that a surfeit of one type of spermatozoa attracted to some eggs sufficiently depletes these spermatozoa elsewhere to produce a reciprocal bias in their capacity to fertilize the alternative eggs (Fig. 1D i). On the other hand, when the spermatozoa are evenly distributed, the facility with which some spermatozoa penetrate half of the eggs would not affect the segregation of the father's genes in respect of the others (Fig. 1D ii). The passive and active varieties of selective retention are determined according as the gene for which the father is heterozygous confers the selective property on all his spermatozoa, so that both his alleles cause unequal recovery of the mother's genes (Fig. 1D iii), or only on that half to which the gene itself is transmitted, when the maternal genes are recovered normally in zygotes derived from the other spermatozoa (Fig. 1D iv). It is interesting to note that paternal influences on spermatozoa are considered by Braden & Gluecksohn-Waelsch (1958) to be responsible for the infertility of viable *tt* males.

The types of selective fertilization have been depicted separately merely as a descriptive convenience, and once alleles find phenotypic expression in gametes there seems no reason why selective gametogenesis, transport and conjugation should not concur. Indeed, the results presented in the later sections of this paper

show that besides the anomalies in the segregation of t^e that are attributed to selective transport, other anomalies are only accountable in terms of selective penetration.

3. MATERIAL

Mice used in the investigation into selective conjugation in respect of the T-locus were descended from a brachyury stock (B stock) and the laboratory stock (L stock) in which the t^e gene was discovered. The supply of Tt^e, $T+$ and $+t^e$ animals required by the investigation was obtained from matings between Tt^e mothers and $T+$ fathers. These, being F_1's or backcross hybrids to the L stock, were either one-half or three-quarters L stock, whereas the experimental animals were one-half or five-eighths L stock as at least one of their parents was an F_1. This material was the more homogeneous as the t^e and + genes always derived from the L stock and only the T gene derived from B stock.

4. METHODS

It will be remembered from the earlier results reviewed in this Introduction that the lethal gene t^e was transmitted by heterozygous males in unusually high frequency to their offspring in general, but that this phenomenon alone (believed to be due to selective transport of t^e spermatozoa to the site of fertilization) could not entirely account for the frequency of lethal heterozygotes in the original population. It was thought that the discrepancy might be made good if t^e spermatozoa, after reaching the site of fertilization, then selected the eggs with which they conjugated. Reference to Fig. 1D i–iv shows that evidence on selective conjugation may be obtained when parents, both of which must be heterozygotes, are chosen in such a way that four zygotic classes are distinguishable in their offspring. In this experiment expected frequencies of the zygotes are written in terms of s (respecting zygotes derived from selective fertilizations by t^e spermatozoa) and r (from the residual fertilizations by spermatozoa carrying an alternative allele). Both s and r are given subscripts T, + and t to designate the allele provided by the egg. This symbolism does not presume specific types of fertilization, but we shall use it solely to gain evidence on selective conjugation, as it is unnecessary to reduplicate the evidence on other types of fertilization at this locus. Figure 2 depicts the results expected from each form of selective conjugation for two types of mating actually made. The expectations are seen to be complicated both by the inequality of the two classes of spermatozoa reaching the site of fertilization, and by the inconstancy of their relative sizes between one mating and another (Smith, 1956; Braden, 1958). Further reference will be made to this figure when interpreting the results of these matings.

Data on selective conjugation could be obtained from three kinds of heterozygous females (Tt^e, $T+$ and $+t^e$) in matings to two kinds of males heterozygous for t^e (Tt^e and $+t^e$). However, out of the six possible types of mating only three (numbered types 4–6 in Tables 2–5) were made, as the others gave less than four distinguishable classes of zygote. Unfortunately, the death of either TT or t^et^e embryos in each

mating left only three recordable classes at birth. Though these were adequate for general tests of selective conjugation, its nature could not be determined from these matings alone. Consequently, special control matings (type 3) were made where,

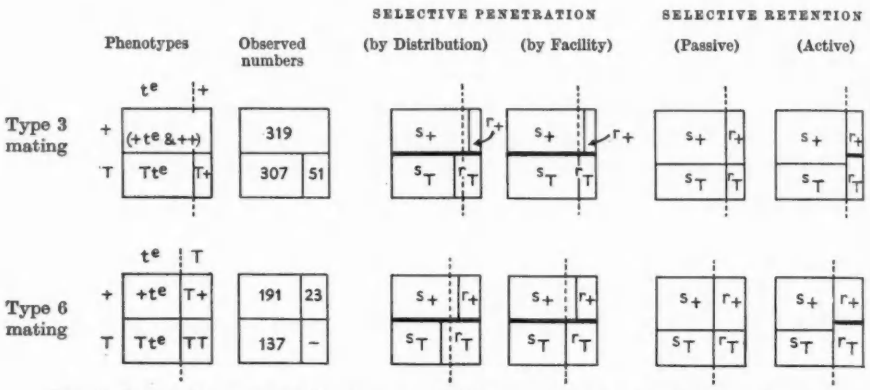


Fig. 2. Interaction of selective transport of t^e spermatozoa with the four forms of selective conjugation: consequences for offspring of $T+$ females mated with $+t^e$ and Tt^e males. The dotted vertical line represents the frequencies in which t^e and $+$ or T spermatozoa reach the site of fertilization.

although two of the products of conjugation were indistinguishable, all four products survived.

Finally, two other control matings were put up to give information on the extent of phenotypic overlapping and on the viability of Tt^e, $T+$ and $+t^e$ zygotes, for

Table 2. *Expectation of progeny in control and experimental families*

Type	Parents ♀ × ♂	Progeny		
		Tt^e	$T+$	$+$
	Controls			
1	$Tt^e \times T+$	1	1	1
2	$+t^e \times T+$	1	1	2
3	$T+ \times +t^e$	s_T : 1	r_T	$s_+ : r_+$: 1
	Experimentals			
4	$Tt^e \times +t^e$	s_T	r_T	r_t
5	$+t^e \times Tt^e$	r_t	r_+	s_+
6	$T+ \times Tt^e$	s_T	r_+	s_+

without this information the frequencies of gametic conjugations in experimental matings could not be properly assessed. Control matings types 1 and 2, using Tt^e and $+t^e$ females respectively, were expected to yield their offspring in Mendelian ratios. It so happened that type 3 matings, using $T+$ females, provided no evidence

of selective retention and could therefore be regarded as controls, too. In this way, control and experimental matings used females of identical genotypes as regards the T-locus. Similarity of genetic background was assured by allocating both sisters and brothers equally to the two groups of matings. Expectations of progeny for the two groups are set out in Table 2.

Between six and twenty-five matings were made of each type. Post-natal inviability of Tt^e mice made it difficult to obtain many matings using such females. Offspring were scored on their day of birth as having zero, short or normal tails, and were discarded. The dams were then re-mated. As many litters were raised as were possible during the useful reproductive lives of the parents. Parents that gave rise to aberrant progenies were finally bred to other mates to ensure that no errors had been made in classifying parental genotypes.

5. RESULTS

Type 1 and 2 control families

Matings of types 1 and 2 were made as controls on viability and phenotypic overlapping. Progeny totals and expectations are given in Table 3. The segregations are perfectly normal and are consistent among individual families (chi-squares for

Table 3. *Segregation in control families*

	Parents	Progeny						
Type	♀ × ♂	Tt^e	$T+$	$+$	Expected ratio	χ^2	d.f.	P
1	$Tt^e \times T+$	74	74	72	1 : 1 : 1	0·04	2	0·98
2	$+t^e \times T+$	199	175	388	1 : 1 : 2	1·77	2	0·45
3	$T+ \times +t^e$	307	51	319	s_T : r_T : 1	2·25	1	0·15
		358		319	1 : 1			

heterogeneity were $\chi^2_8 = 1{\cdot}52, P = 0{\cdot}99$; and $\chi^2_{24} = 21{\cdot}96, P = 0{\cdot}60$). It is concluded that tailless, short-tailed and normal-tailed foetuses were neither unequal in viability nor subject to serious phenotypic overlapping. (Indeed, only one case of phenotypic overlapping occurred among 211 individuals whose genotypes were definitely known. This case was due to a genetically short-tailed mouse who had the appearance of a tailless one.) These conclusions strictly apply to offspring of tailless and normal-tailed females from whom the data were gathered. However, it would be fair to assume that the situation would be the same for the remaining short-tailed class of experimental females that are both phenotypically and genotypically intermediate. It is evident that the disturbed segregations among the progeny of $+t^e$ and Tt^e fathers are wholly consequent upon selective fertilization.

Type 3 control families

Type 3 control matings were made to determine whether irregular conjugations from heterozygous t^e males could be attributed to selective retention, as would have been indicated if maternal genes in this mating had segregated abnormally. But the

maternal genes are roughly equally represented (Table 3), and the majority of constituent families agree in this ($\chi^2_{10} = 16{\cdot}62$; $P = 0{\cdot}09$).

However, this is not highly critical evidence that selective conjugation with t^e spermatozoa was by selective penetration, and it is desirable to extend the field from which evidence may be drawn. Accordingly we shall now attempt to compare segregation in matings of types 3 and 6 (Fig. 2), where different heterozygous t^e males (Tt^e and $+t^e$) were used on the same ($T+$) females. In the case of passive selective retention it is clearly meaningful, no matter how $s : r$ ratios differ between mating types, to compare the numbers of $+$ and T eggs fertilized by all spermatozoa in type 3 matings with the numbers fertilized only by t^e spermatozoa in type 6. The expectation is that $(s_+ + r_+)/(s_T + r_T)$ for type 3 $= s_+/s_T$ for type 6. There is practically the same expectation from active selective retention considering 86% eggs were fertilized by t^e spermatozoa. On the other hand, in the case of selective penetration there is neither reason why the two sides of the above equation should have similar values, nor any alternative meaningful comparison to be made. Consequently, contingency of the two sets of figures (319 : 358 for type 3, and 191 : 137 for type 6) would be evidence of selective retention. However, $\chi^2_1 = 10{\cdot}91$, $P = 0{\cdot}001$; and the obvious lack of contingency firmly supports the previous evidence of selective penetration. It is inferred that wherever selective conjugation is encountered in the experimental matings it will be the result of selective penetration. Arising from this conclusion is the practical point for interpreting the experimental findings: the larger of two r-values from a family implies a smaller value of the corresponding s. This reciprocal relation does not hold for selective retention.

Experimental families

Data on the progeny totals for the experimental families are given in Table 4. In each there is an expectation that two of the progeny classes will occur approximately equally (both having the expectation s, or both r) whose equality over all families of the same type can be statistically tested. The results of these tests are given in Table 4. Uniformity tests were also made to determine whether the pairs of s-values or the pairs of r-values bore the same relation to each other within each family as in the type as a whole. These yielded the following χ^2's: $\chi^2_5 = 3{\cdot}64$, $P = 0{\cdot}60$ (type 4); $\chi^2_{11} = 22{\cdot}17$, $P = 0{\cdot}02$ (type 5); $\chi^2_8 = 9{\cdot}30$, $P = 0{\cdot}30$ (type 6). Families of type 5 were the only ones to conform to expectation as a group, but were also the only ones among which there was significant heterogeneity. Thus, in some matings of this type, and in general for matings of the other two types, the number of eggs fertilized by t^e

Table 4. *Segregation in experimental families*

	Parents	Progeny				Equality of s- or r-values		
Type	♀ × ♂	Tt^e	$T+$	$+$	Expected ratio	Comparison	χ^2_1	P
4	$Tt^e \times +t^e$	164	23	43	$s_T : r_T : r_t$	$r_T : r_t$	6·06	0·013
5	$+t^e \times Tt^e$	67	54	155	$r_t : r_+ : s_+$	$r_t : r_+$	1·40	0·24
6	$T+ \times Tt^e$	137	23	191	$s_T : r_+ : s_+$	$s_T : s_+$	8·89	0·005

spermatozoa varied with the constitution of the eggs, although the alternatives were provided by the same female. In other words, conjugation was selective. Having already dismissed the possibility of selective retention because of the results of type 3 control matings, the manner of conjugation here was presumably by selective penetration.

The matter can be examined more closely by studying individual families. As none was large, no single family could be expected to give statistically conclusive results; yet it is not desirable to pay attention to results that were almost certainly due to vagaries of chance. Somewhat arbitrarily, therefore, further attention is confined to those families whose ratios departed far enough from expectation that their likelihood of occurrence by chance was less than one-fifth ($P \leqslant 0{\cdot}2$). Of twenty-seven experimental families, nine were in this class, and the information from these

Table 5. *Segregation in individually aberrant families of progeny expected in equal numbers*

Type	Parents ♀ × ♂	Expected relation	Progeny Tt^e	$T+$	$+$	Conclusion
4	$Tt^e \times +t^e$	$— : r_T : r_t$	—	9	21	$s_T > s_t$
			—	8	16	$s_T > s_t$
5	$+t_e \times Tt^e$	$r_t : r_+ : —$	4	0	—	$s_+ > s_t$
			0	5	—	$s_+ < s_t$
			13	2	—	$s_+ > s_t$
6	$T+ \times Tt^e$	$s_T : — : s_+$	9	—	18	$s_+ > s_T$
			0	—	4	$s_+ > s_T$
			28	—	47	$s_+ > s_T$
			23	—	36	$s_+ > s_T$

is set out in Table 5. (Irrelevant phenotypes are not included.) It is observed that, with only one exception, the relative values of the s's throughout the three types of mating fell into a consistent pattern; namely, $s_+ > s_T > s_t$.

The following conclusions are drawn.

1. The great majority, about 84%, of effective spermatozoa from Tt^e and $+t^e$ males transmitted t^e.

2. Maternal genes in matings of type 3 ($T+$♀$\times +t^e$♂) were equally represented in the progeny and neither gene was selectively retained in the egg nucleus. Unfortunately, as two of the classes of progeny were phenotypically alike ($+t^e$ and $++$ are both normal-tailed) these matings provided no direct information about selective penetration.

3. In other matings with heterozygous t^e males, in which three products of conjugation were distinguishable and the fourth lethal, the one genotype of spermatozoa that formed viable combinations with both eggs conjugated with different numbers of them. The missing lethals did not admit direct evidence on the kind of conjugation, but as type 3 families did not display selective retention, it is presumed that these anomalous conjugations involved selective penetration.

4. In this case, since maternal alleles enter the fertilized eggs equally, $(s_T + r_T) = (s_+ + r_+) = (s_t + r_t) = \frac{1}{2}$. Using the data of Table 4 to estimate the numbers of

conjugations of t^e spermatozoa with preferred and avoided eggs, it appears that 55% of t^e conjugations were with preferred eggs, 45% with avoided eggs.

5. The T-locus is probably near a centromere. This conclusion follows from the greater likelihood that portions of chromatids are exchanged between bivalents as distance from the centromere increases. For any included locus, each exchange in oogenesis means that heterozygous alleles will be conveyed together into the products of the first division, and that another egg will be heterozygous when uniting with the spermatozoon. Thus there are few opportunities at distal loci for penetration to be selective. On the other hand, the absence of exchanges proximal to the centromere ensures complete segregation of alleles in this region, complete differentiation of the eggs, and every opportunity is given to selective penetration.

6. DISCUSSION

The Mendelian expectation of offspring from heterozygous parents is 1 : 1 : 1 : 1. T, + and t^e segregate from females, and T and + (but emphatically not t^e) segregate from males to give progenies agreeing closely with this ratio. Whereas most departures from Mendelian expectation are attributed to phenotypic overlapping or irregular viability, and underlying gametic ratios are assumed to be perfect, this hypothesis is precluded from explaining anomalous segregation of t^e from males. Thus, when heterozygous t^e males are avoided, segregation is perfect for genetically similar offspring to theirs. Gametic anomalies are therefore implicated, and two have been found.

First, t^e is transmitted by spermatozoa of heterozygous males in far greater frequency than either allele. The consequences of this well-known phenomenon have been fully discussed for t genes in general by Dunn (1953) and for the t^e gene in particular by Bateman (1960). It need only be noted here that delays in mating so profoundly affect the transmission of other t alleles (Braden, 1958) that it cannot reflect the numbers of spermatozoa at gametogenesis, and is most likely produced by selective sperm transport inside the female genital tract.

Second, the new observation was made that unequal numbers of t^e spermatozoa fertilized alternative eggs of heterozygous females. It is remarkable that this unique test of the randomness of gametic conjugation conflicts with that usually accepted assumption. However, until selective conjugation is described for other t alleles as well, its occurrence here cannot begin to have general significance.

Nevertheless, the selective conjugations of t^e spermatozoa are particularly relevant to the previously unexplained frequency of $+t^e$ animals in the original laboratory stock (Bateman, 1960). Mice were of two kinds: homozygous normals (+ +) and heterozygotes ($+t^e$). In matings with $+t^e$ males, the proportion, P, of t^e spermatozoa among those partaking in fertilization was estimated to be 0·87, from which it was expected that 0·62 animals would be heterozygous. The actual figure was much higher than this, and was 0·80. These figures were highly discrepant ($P \ll 0{\cdot}001$). However, it was not then realized that the description of fertilization was incomplete when stated in terms of the overall parameter P and that it was necessary to know the frequencies in which each zygote was formed. Hence the

current description in terms of selective conjugations between t^e spermatozoa and + eggs (s_+), t^e spermatozoa and t^e eggs (s_t); and of residual conjugations between + spermatozoa and + eggs (r_+), and + spermatozoa and t^e eggs (r_t). Previously, the frequency of t^e's entry into zygotes via spermatozoa was judged from fertilizations of T eggs ($P \equiv s_T$), but present evidence shows that t^e spermatozoa made more zygotes with + eggs and fewer with t^e eggs ($s_+ > s_T > s_t$). Both departures would conserve the t^e gene and raise the expected number of heterozygotes. Indeed, it is probable from conclusion 4, that t^e spermatozoa fertilized 10% more + eggs than originally estimated (i.e. 96%, instead of 87%), and 10% fewer t^e eggs (i.e. 78%). If these figures are applied to the original population, assuming random mating and 80% heterozygotes, it will be found that 81% of surviving animals of the next generation are expected to be heterozygotes. Thus it is entirely possible to account for the frequency of lethal heterozygotes in the original laboratory population by the observed results of selective transport of spermatozoa and of their subsequent selective conjugation (penetration) with the eggs. One wonders to what extent these are actually related aspects of selective fertilization. It is conceivable that t^e and + (or T) spermatozoa reach the egg cluster in equal numbers, and that t^e spermatozoa are generally superior at getting into the eggs through the enclosing cumulus oophorus, but are especially superior at getting into + eggs. In this case, the distinction between transport and penetration would be extremely subtle. In any case, the high frequency of lethal heterozygotes in the original laboratory population is accounted for in terms of gametic selection alone without recourse to wide variations in parental fitness other than implicated by the death of $t^e t^e$ offspring. Usually in animal populations gametes are credited with replicating genes perfectly in the same frequencies that they occurred in the parents, and selection is regarded as wholly zygotic. The position taken by Dunn and his collaborators (Dunn, 1953; Dunn & Gluecksohn-Waelsch, 1953; Dunn & Suckling, 1956; Dunn, 1957; Dunn, Beasley & Tinker, 1958) regarding the incidence of tailless-lethals in wild populations is intermediate in the sense that they are looking for explanations using both gametic and zygotic selection.

SUMMARY

Advantage was taken of a unique situation to test, with adequate controls, the assertion that male and female gametes conjugate at random. The data concern the aberrant locus T of the mouse, at which there are a number of *t* alleles that enter into the majority of effective spermatozoa of males heterozygous for one of them. Segregation in females is normal. Evidence is presented for one of these *t* alleles, tailless-Edinburgh (t^e), that conjugation between gametes was not at random when this gene was present in spermatozoa. When a choice of eggs was presented by heterozygous females, tailless-Edinburgh spermatozoa united more frequently with normal than with brachyury eggs and more frequently with brachyury than with tailless-Edinburgh eggs. The relevance of this finding is discussed in relation to expected equilibria of *t* alleles in closed populations. Other forms of selective fertilization are discussed and their genetic consequences compared.

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The effects on litter size of crossing lines of mice inbred without selection

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1. INTRODUCTION

In the exploitation of heterosis in plant and animal improvement, inbreeding is frequently employed to produce genetic differentiation in the parent material, through random changes in gene frequencies. But if the resultant crosses are to represent genetic progress, random differentiation alone becomes insufficient, for genotypes of special merit cannot be provided without selection at some stage. The selection, which may be either natural or artificial, can apply or be applied at any of these stages:

1. Selection within lines on inbred performance.
2. Selection between lines on inbred performance.
3. Selection between lines on crossing performance (general combining ability).
4. Selection between crosses on cross performance (special combining ability).

Natural selection will act primarily through the first two ways, if the character is an aspect of natural fitness. Artificial selection can also be applied during inbreeding, though theoretically it is best reserved until the crossing programme.

Little, however, is known about the efficacy of selection in the context of inbreeding, and the experiment to be described here was designed to provide information relevant to this general problem. The character studied was litter size in the mouse. The results are therefore relevant to some problems in animal improvement, especially to such characters as the fertility of pigs.

The general plan was to inbreed a number of lines without any artificial selection, and with minimal natural selection. To this end, it was imperative to preserve all possible lines. This in turn precluded raising the inbreeding coefficient above 50% or so, for by previous experience the loss of lines then becomes inevitable. Thus the experiment was of necessity restricted to only partly inbred material, but the obvious theoretical disadvantage of this was somewhat mitigated by greater practical application. For the difficulty and cost of maintaining inbred lines becomes prohibitive in farm animals, even in pigs (see, for instance, Donald, 1955), so that the use of partly inbred material must be explored.

The lines were crossed to obtain the following information:

(*a*) To compare the performance of the crossbred population with that of the original outbred population from which the inbreds were derived. This comparison would indicate what improvement, if any, would accrue from natural selection which operated almost entirely within lines (1 above).

(*b*) To estimate the variances of general and special combining abilities. These estimates would assess the effect of artificial selection applied in the manner of 3 and 4 above.

The application of selection in the manner of 1 and 2 above was the subject of another experiment, on the same stock of mice, described by Bowman and Falconer (1960).

2. THE CHARACTER—LITTER SIZE

Litter size would appear to be a self-explanatory term—the number of young born in a litter. This definition is unfortunately complicated by the disposition of mice to eat many of their still-born young—and possibly some others as well. The number of young found is thus influenced by the interval between birth and the examination of the litter. In the experiment reported here, cages were examined once daily, the number of live young being recorded as the litter size.

All the work was done on first litters only. The collection of sufficient information on second litters to be of material assistance would inordinately prolong the generation interval, sufficiently so as to nullify the advantage of more accurate measurement. The character chosen for study was therefore 'the number of live young found in the first litter'. While this may not reflect accurately the common concept of 'litter size', the term as defined has complete operational validity.

Litter size as a character is one of considerable complexity. It has three major factors, each of which determines the upper limit of the succeeding one:

1. The number of ova shed.
2. The number of ova fertilized.
3. The number of zygotes carried to term.

The first of these is of course wholly a character of the dam. The second may be influenced by either the sire or the dam. Though Falconer (1955) showed the effect of the sire on litter size in outbreds to be negligible, this may not be so in an inbred population. While it may be tempting to regard the third component as a function of the viability of the young, we cannot exclude the potential influence of the dam, quite apart from her contribution to the genotype of the litter. It can be seen, therefore, that when litter size as a character is submitted to any genetical analysis, its constituent factors are intricately confounded. This problem will be discussed at greater length when the actual results are examined.

The complexity of the character, however, does not end with its multiple determination. For the number of young born is subject to a strong maternal effect dependent upon the weight of the mother. A large mother tends to produce a large litter, in which individual weights are consequently depressed. This handicap is still reflected in weight at mating time. Hence the daughter of a large mother tends to be light, and produces a small litter when she in turn bears offspring. The net effect is thus a negative regression of litter size on the size of the litter in which the dam was born, unless there also exists the positive genetic pathway expected of a heritable character. These complicated interactions were studied by Falconer (1955), who calculated the path coefficients relating litter size to the body weight of

the dam and the size of the litter in which the dam was born. The path diagram is shown in Fig. 1. The mother's body weight is inversely correlated with the size of the litter in which she was born, and directly with the size of her own litter. The product of these two coefficients is $-0{\cdot}07$, which would give the regression coefficient of litter size on maternal litter size if no other pathway were operative. There is, however, a direct genetic pathway, which is measured as the partial regression of litter size on maternal litter size holding the mother's weight constant. This coefficient is $+0{\cdot}07$, as shown. From this, we see that litter size is affected by maternal

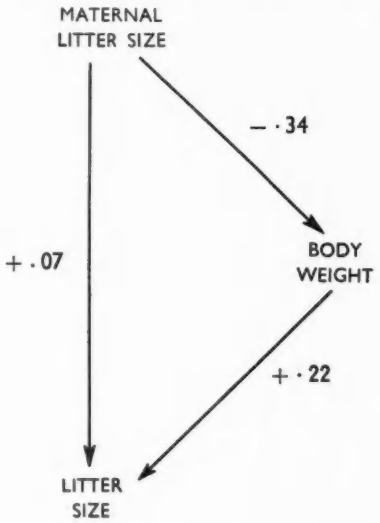

Fig. 1. Path diagram and standardized partial regression coefficients. After Falconer (1955).

litter size through two independent pathways of equal magnitude but opposite sign, explaining why the direct regression, when measured, comes out to be zero (Falconer, 1955).

The relevance of these maternal effects to the subject of this paper is apparent from the following considerations. Firstly, as litter size declines on inbreeding, a maternal effect will be initiated counteracting to some extent the direct effect of inbreeding. But body size itself, and other characters such as milk production, are also liable to be depressed by inbreeding. The possible interactions become so involved that the nett effect becomes obscure. At the present state of our knowledge, we can only approach the problem empirically, and this discussion of the complexity of the character has been presented to show that any attempts at a more sophisticated interpretation of the subject would only be of questionable validity.

3. MATERIALS AND METHODS

It was a premise of the experiment that no lines should be lost during inbreeding. Previous experience had shown that in practice it would be impossible to carry the

inbreeding coefficient beyond about 0·50 without introducing the likelihood of losing lines through low fertility or even complete sterility. It was clear from the start that the crossing would have to be done from partly inbred material.

The broad outline of the experiment was therefore as follows. The inbreeding stage was confined to three generations of brother-sister mating. The lines were then crossed at random giving crossbred litters. As litter size is largely a maternal character, these crossbreds had to be mated to test their fertility, for this was what the experiment was required to determine.

(i) *Inbreeding programme*

It was decided to start with thirty inbred lines, which were derived from mice surplus to the requirements of a selection experiment for litter size described by Falconer (1955). Ten inbred lines were derived from each of the high, low and control stocks of the selection experiment, which had then proceeded for ten generations. There was therefore some differentiation among the original material which had to be allowed for in the crossing programme. Ten litters were chosen from each stock; each litter came from one family and subsequently became the foundation of one inbred line. The largest and smallest litters in the 'high' and 'low' stocks respectively were of course required for the selection experiment. In choosing litters for the present work, this bias was counteracted by rejecting also the other extreme. With this exception, and the avoidance of sib litters, the foundation litters were taken at random.

The inbred lines were propagated in the following manner. All the available females of a litter were divided between two of their sib males, as a precaution against male sterility or accidental loss. Each line thus normally gave birth to more than one litter, one of which was taken at random. The random choice was occasionally disturbed by a litter not containing the required two males and two females, which was usually excluded in the interest of safeguarding the line. But any selection thereby introduced against litters of extreme sex ratio and against some small litters was so slight (and probably ineffective) that it was considered to be of little consequence.

The mice were mated when the youngest reached 6 weeks of age, the oldest mice of that generation being approximately 8 weeks by that time.

In spite of all reasonable efforts to maintain them, four lines in fact failed to complete the inbreeding stage of the experiment, and of course are not represented in the crosses. Two lines were lost for reasons unconnected with fertility, but the loss of the other two must be ascribed at least in part to low fertility. Each gave birth to small litters, all of which died before weaning. There was therefore undoubtedly a little selection during inbreeding, but its magnitude must be considered insufficient to affect materially any conclusions that emerged from the work.

(ii) *Crossing programme*

Ideally, each line should be crossed to all the other lines to form an orthogonal set of diallel crosses, but this was prevented by the exigencies of space. Any system

whereby the crossing was done at random would meet the basic requirements of the experiment, and the principle of the scheme finally adopted is illustrated in Fig. 2. In order to use all available lines as both male and female parents, pair-matings were employed. The size of the litter of any one pair was an estimate of the value of that cross. A certain number of replicate crosses was therefore required to assess the error variance.

All crossing was done within each of the three major groups from which the inbreds were derived. The scheme depicted in Fig. 2 was therefore used for each

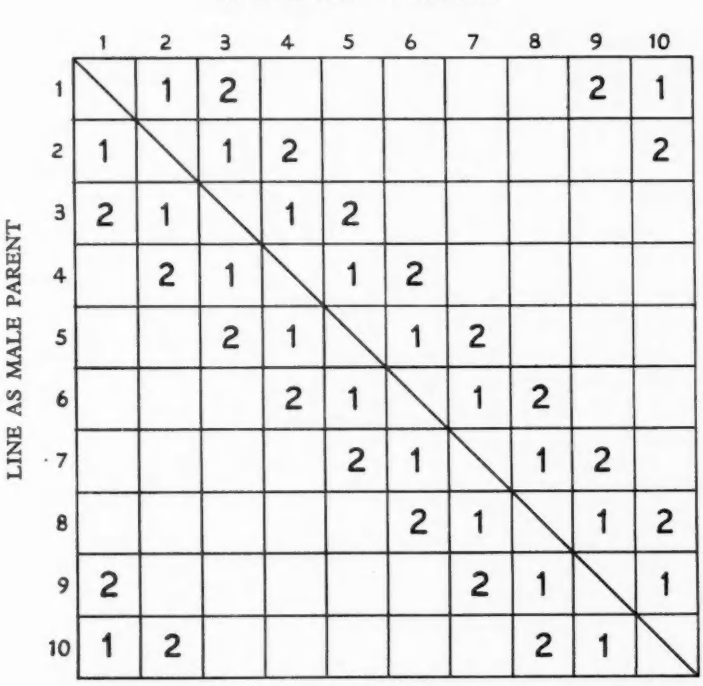

Fig. 2. The principle of the scheme of crossing the inbred lines. The number in each cell represents the number of matings between those lines.

group in turn. There were insufficient mice available to make all the matings required by this general scheme, but when a particular mating could not be made no other was substituted. This would introduce the least bias into the crossing programme.

The crossing programme required two stages, one to obtain the crossbred animals and another to test their fertility. The first cross measures the effect on litter size of crossbreeding in the litter, but still from an inbred mother. The second cross measures the further effect on litter size brought about by using a crossbred mother. In the second cross, litter size is regarded as a maternal character, as the direct effect of the male on litter size in fertile outbreds was known to be negligible (Falconer,

1955). We are interested in the effect on litter size of the genotype of the mother, as determined by her inbred parents.

In the second cross, matings between crossbred mice with a common parental line was avoided. Apart from that, the mice were mated schematically as before. The scheme of crossing employed was in principle a repeat of the first cross, except that a certain number of triplicate matings were substituted for the duplicate ones of the first cross. This was done as the error variance in the first cross was rather large.

As the inbreeding of parents and offspring are out of step throughout the experiment, Table 1 shows the inbreeding coefficients of parents and of offspring for every generation. The foundation animals are designated generation O, the inbred generations I, and the crosses X.

Table 1

Generation	Inbreeding coefficient: Parents	Inbreeding coefficient: Offspring	Litter size	Body weight of dam at 6 weeks (g.)
O	0	0	8·12	21·9
I_1	0	0·25	6·73	21·2
I_2	0·25	0·375	5·82	20·8
I_3	0·375	0·50	5·69	20·1
X_1	0·50	0	6·20	21·5
X_2	0	0	8·47	21·3

4. RESULTS

The data that accrued from the experimental work will be presented in three sections in the following order:

(*a*) The effects of inbreeding and crossing on mean litter size.
(*b*) The differentiation between inbred lines in litter size.
(*c*) The analysis of variance of litter size in crosses between inbred lines.

(*a*) *Mean litter size*

To a limited extent, it is possible to observe separately the effect on litter size of inbreeding in the dam and inbreeding in the litter. In the first inbred generation, any reduction in litter size is clearly attributable to inbreeding in the young, as the parents are still outbred. Likewise, any increase in the first cross will be due to crossbreeding in the litter, and any further increase in the four-line crosses can be ascribed to crossbreeding in the parents. But, for the intermediate generations of the experiment, the inbreeding of parents and young will proceed simultaneously but at different stages.

The generation means for litter size during the inbreeding and crossing phases of the experiment are shown in Table 1, and are illustrated graphically in Fig. 3. The general picture is the expected one of decline on inbreeding, with subsequent recovery on crossing the inbred lines. In the first generation of inbreeding, mean litter size fell by 1·39 as a result of increasing the inbreeding coefficient of the young from

0 to 0·25. Over the next two generations, there was a further fall of 1·04 in mean litter size; as indicated earlier, it cannot be determined to what extent this is due to further inbreeding in the young, and to what extent it is caused by inbreeding in the parents.

In the first crossbred generation, when the inbreeding coefficient of the young was changed from 0·50 to 0, litter size improved by 0·51. This, of course, is a minimal

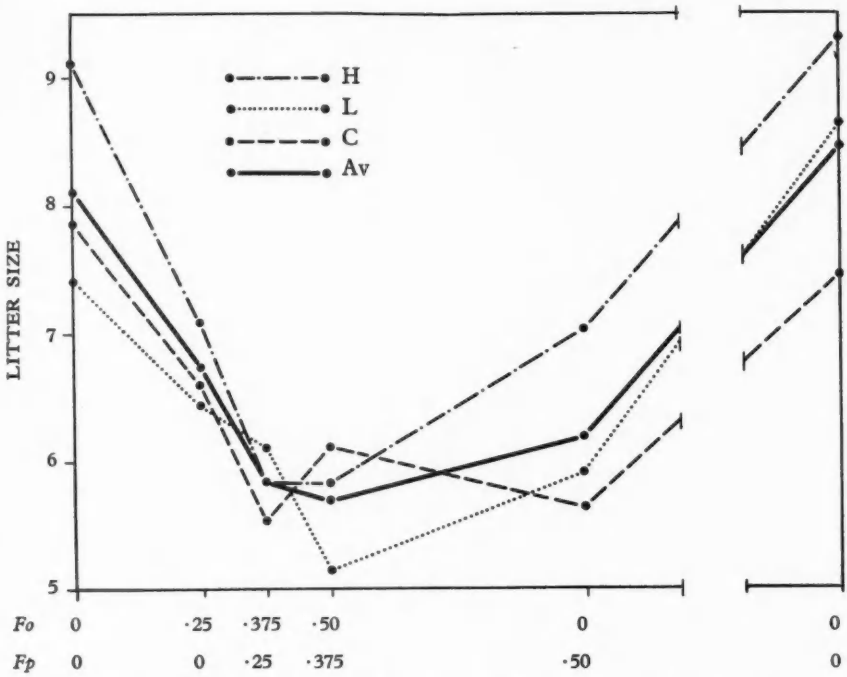

Fig. 3. Litter size plotted against inbreeding coefficient of the offspring (F_o). Inbreeding coefficient of parents (F_p) also shown. Group H—previously selected for high litter size; Group L—previously selected for low litter size; Group C—not previously selected; Av—average of all groups.

estimate of the initial effect of crossbreeding. Had the inbred parents borne inbred rather than crossbred young, their estimated litter size would be somewhere in the region of 5·0, assuming a linear decline. This would indicate that the real effect of crossbreeding in the litter was to increase litter size by rather more than one mouse. Nevertheless, this increase was considerably smaller than expected if we were to extrapolate from the results of the first inbred generation, where a bigger change in litter size occurred for only half the change in inbreeding coefficient. The anomaly does not end here. In the second inbred generation, the additional effect of inbreeding in the dam was barely perceptible over the expected effect of further inbreeding in the young. Yet, in generation X_2, the effect of crossbreeding in the dam was to

increase litter size by 2·27 over the previous generation. The data suggest that inbreeding may impose a limit on the dam's potential fertility, and that no amount of heterozygosity in the young would increase litter size above a certain level. In outbred dams, on the other hand, any reduced viability through inbreeding in the unborn young would be fully revealed in the reduced litter size at birth.

The mean litter size of the crossbred mice in generation X_2 is 0·35 of a mouse higher than in the original outbreds, generation O. The comparison of these two means is of prime importance and represents one major interest of the experiment. The difference is not significant at the 5% level, despite the slight involuntary between-line selection during inbreeding, mentioned earlier. Over the period of the experiment, generation means of the outbred control varied between 7·00 and 8·17, which makes a difference of 0·35 appear unimportant. There is therefore no reason to suppose that natural selection operating within lines during inbreeding has had any effect on the mean performance of the derived crossbred population.

It is perhaps of some interest to consider separately the three groups of mice from which the inbred lines were derived. These groups, though initially of common origin, had become differentiated through selection for high and low litter size, the third group being an unselected control. It is conceivable therefore that the effect of inbreeding on litter size could well be different in the different groups. Considering first the two groups that had previously been selected, it seems that after three full-sib matings, litter size had declined in both by approximately the same proportionate amount to about two-thirds of the initial litter size. But the increase on crossing was relatively greater in the group erstwhile selected for small litters. However, the standard errors of all these estimates of group means were of the order of 0·4 of a mouse, and without any more elaborate statistical analysis it is clear that apparent differential trends of the magnitude observed would not be significant. In the group that had not previously been selected, litter size increased during the last generation of inbreeding and fell again when the lines were crossed. This does not accord with expectation nor with the behaviour of the other two groups. It seems probable that the estimate of the mean of the I_3 generation in this group is spuriously high either through sampling errors or through some short-term environmental influence which the other groups did not encounter.

Litter size, as mentioned earlier, is markedly affected by the weight of the dam, and the picture is therefore not complete without the examination of this correlated character for possible changes during the experiment. If weight were to decrease on inbreeding with a subsequent increase on crossing, this would have obvious repercussions on the interpretation of the observed effects on litter size. Because of this possibility, the weight of the females was recorded at 6 weeks, the approximate age at mating. The mean weight is shown in Table 1. The first conclusion is that 6-week weight did not change in any systematic manner with changes in heterozygosity. Secondly, such changes as were observed were so small that any correction of litter size for dam's weight would only have a trivial effect. Though body weight in standardized litters is known to decline on inbreeding, it seems that in this experiment the depression was more or less balanced by the advantage gained

through a simultaneous reduction in litter size. Likewise, when the lines were crossed, the potential increase in body weight was nullified by the increase in litter size.

(*b*) *Differentiation between inbred lines*

The classical theory of inbreeding indicates that inbred lines become differentiated, with a corresponding increase in uniformity within lines. The mathematical expressions for the variances between and within lines are $2F\sigma_A^2$ and $(1-F)\sigma_A^2$, respectively, where σ_A^2 is the additive genetic variance in the initial population and F is Wright's coefficient of inbreeding. At complete inbreeding the initial genetic variation is thereby doubled, and it all appears between lines. However, these expressions are true only if all the variance is additive; they will not hold where dominance and epistatic deviations exist, and in most instances the observed result on inbreeding will differ from expectation based on an additive model.

The theoretical treatment of the effect of inbreeding on variation in a non-additive situation has not been developed fully, but Robertson (1952) has examined the consequences on variation due to rare recessive genes. He showed that the within-line variance in such a case would increase on inbreeding until F is in the region of 0·5 and then decline. The between-line variance will also increase, but only slowly at first as the increase is proportional to F^3. Robertson shows further that the same general conclusions will probably apply to genes showing over-dominance.

It appears therefore that, in an unknown genetic situation, changes in within-line and between-line variances are unpredictable, and for this reason every empirical observation is of some value. The results obtained from the present work are summarized in Table 2. The data from generation X_1, where the offspring are crossbred, are not included as the variance observed cannot be partitioned in a simple manner into within-line and between-line components.

Table 2. *Variance components within and between inbred lines*

	Generation		
	I_1	I_2	I_3
Within-line component	5·19	6·70	3·16
Between-line component	1·49	0·08	2·81

With only three points available for examination, it is clearly impossible to establish any definite trend. Further, as the estimates of the within-line and between-line components are necessarily negatively correlated, it becomes difficult to deduce anything about their interrelationship. The values obtained for the I_2 generation must be spurious, for on no model would the differentiation between lines vanish so suddenly only to re-emerge in the subsequent generation. But if any reliance can be placed on the other estimates, it seems that the total variation is being repartitioned in the direction of increasing the differentiation between lines.

(c) *Analysis of variance in crosses*

The data have to be analysed in two distinct classifications. The first of these concerns crosses (irrespective of whether the cross is AB or BA), reciprocal members of the same cross, and error variance. In the second classification, the variance is partitioned between dam-lines, between sire-lines and the interaction between them. We shall consider the two classifications in this order.

The error variance is of course common to both. This was estimated from twenty-two duplicate crosses in the first cross, and forty triplicate matings in the second. In these replicate crosses, parents of the same sex were always taken from the same line. It proved to be immaterial whether replicates were taken from the same litter or from different litters from the same cross. Both analyses were made within the three major groups that constituted the experimental population. 'Group' refers to a set of lines of common origin. The results of the first analysis are shown in Table 3. There seems to be little evidence of variation between crosses in either generation. This indicates that no effective selection of good crosses from the array of possible ones could be made.

Table 3. *Analysis of variance in crosses*

	First cross			Second cross		
	d.f.	M.S.	*P*	d.f.	M.S.	*P*
Total	106	7·18		146	5·03	
Between groups	2	19·46	$>0{\cdot}05<0{\cdot}10$	2	42·70	$<0{\cdot}001$
Within groups	104	6·94		144	4·51	
Between crosses	44	7·08	$>0{\cdot}20$	38	5·91	$>0{\cdot}50$
Within crosses	60	6·84		106	4·01	
Between reciprocals	38	5·93	$>0{\cdot}20$	26	6·54	$<0{\cdot}01$
Between replicates	22	8·41		80	3·18	

The influence of maternal effects on litter size is illustrated by the significant difference between reciprocals in the second cross. No such difference could be established in the first cross, probably because of the magnitude of the error mean square. The large error variance, especially in the first cross, is a disconcerting feature of the data. This suggests that no precise estimates of the components of variance involved could be obtained, without large-scale experimentation.

The second analysis attempted to partition the variance between lines, used both as male and female parents, and to measure the interaction between them. This should enable us to distinguish between the 'general combining ability' of a line, which can be defined as the average performance of crosses between that line and all other lines, and the 'special combining ability' of a cross, measured by the deviation of the performance of that cross from the expectation based on the general combining abilities of its parent lines. The variation in the general combining ability of lines will be represented by the sum of two components of variance, that

between dam-lines and the one between sire-lines. The variation in specific combining ability will be the interaction component of variance.

The method whereby the components were estimated was somewhat complicated, owing to the non-orthogonality of the system of crossing and also because a dam-line was crossed only to some of the sire-lines, and vice versa. The analysis is therefore not presented in any detail, but the principle involved is explained by Henderson (1953). The estimates obtained for the components in the two generations of crossing are shown in Table 4.

Table 4. *Components of variance of litter size in crosses*

Component	First cross	Second cross
Between sire-lines	0	1·08
Between dam-lines	0	0·36
Interaction	0	0·07
Error	8·41	3·18

In the first cross, all the components except error took a small negative value, giving zero as the best estimate in each case. In the second cross, the interaction component was very small indeed, indicating that, in this particular situation, specific combining ability is practically non-existent, and certainly very small compared with the general combining abilities of the lines. Because of their composition, the appropriate mean squares could not be adequately tested for significance level.

The order of magnitude of these components compared to the error variance again indicates that for accurate estimation the scale of the experiment is inadequate. But even after allowing for large error variance, there seems to be little evidence of any useful variation between crosses, indicating that selection between crosses would be ineffective.

5. DISCUSSION

The interpretation of the experimental data has been rendered somewhat imprecise by the complexity of the character of litter size. The difficulties involved can be attributed in no small measure to the dual genetic determination of the character, as the relative contributions of the dam and of the litter itself are seldom clearly distinguishable. In addition, we have strong maternal effects on litter size, and their interplay with inbreeding depression adds further intricacies. The examination of the underlying genetic situation will therefore be severely limited in its scope until such time as the constituent factors of litter size are more perfectly understood.

To some extent we have seen the genotype of the dam and the genotype of the litter acting on litter size separately. At the commencement of inbreeding, reduced viability of the unborn litter had a marked effect which was only partly recovered when the lines were crossed. By then, crossbreeding in the dam appeared to be of

predominating importance in increasing litter size, but the effect of inbreeding in the dam, when first introduced, had been barely perceptible. The explanation may be, in part at least, a maternal effect restricting litter size in inbred mothers irrespective of the heterozygosity of the young. The elimination of lethals with a heterozygote advantage cannot be invoked, for untimately the mean litter size of the original outbred population was restored when the crossbred mice were used as parents. In the absence of selection, this is what theoretical considerations lead us to expect, for unselected inbred lines could then be regarded as a sample of the gametes of the original outbred population. A random cross would therefore represent one individual of the original outbreds.

It is only fair to admit that the apparent contradiction mentioned above could have arisen if the mean litter size for either the I_2 or X_1 generation had been spuriously low. Yet, this seems unlikely, for other workers report analogous findings. Eaton (1953) noted when he crossed inbred lines of mice that the effect on litter size of crossbreeding in the dam was much larger than the effect of crossbreeding in the litter, if inbreeding had proceeded for less than six generations. The assessment of the other point, namely the I_2 generation, is confirmed by Bowman and Falconer (1960), who with the same stock of mice in the same laboratory found a similar rate of decline on inbreeding. When all these complementary phenomena are considered together, the possibility of sampling error becomes reduced, and it would seem that the decline in litter size is not linearly related to inbreeding when its effect in the dam and in the litter are considered separately.

The effect on crossbred performance of any natural selection operating within lines during the inbreeding stage appears now to be unimportant. The improvement in fertility normally associated with crossing subsequent to inbreeding must therefore be ascribed to some other form of selection, as a result of which many of the poorer genotypes would not be represented in the crossbred population. Hybrids between a random array of inbred lines have no intrinsic merit except to the extent that the population was selected during inbreeding. For certain characters, inbreeding and crossing may well provide means of rapid selection, whether natural or artificial, that might not otherwise be possible. Apart from this possibility, the only advantage of the system would be the ability to replicate any desired cross at will.

The lack of variation between the means of the crosses was somewhat unexpected, for the inbred lines were clearly differentiated in the last generation of inbreeding. The probable explanation lies in the use of partly inbred material. It may be shown from a paper by Robertson (1952) that the expected variance between the means of line crosses is $F\sigma_A^2 + F^2\sigma_D^2$, where F is Wright's coefficient of inbreeding when the lines are crossed, σ_A^2 is the additive component of variance, and σ_D^2 is the variance due to dominance. Hence, in this particular experiment only half the additive and a quarter of the dominance variance was available. The additive genetic component of variance in this stock of mice is of the order of 1·5. No similar estimate can be made of the variance due to dominance, but only in special circumstances would it be much greater than the additive component (see, for instance, Mather, 1949). It

is apparent therefore that compared with the error variance observed, these estimates of the genetic sources of variation to be expected, when divided between two generations, become very small. This indicates that before any useful selection could be made between crosses, not only should the experiment be on a larger scale, but also the level of inbreeding should be advanced well beyond 50 per cent.

It can also be shown, from Robertson's paper, that the term $F\sigma_A^2$ represents the component of variation due to the general combining ability of the lines, while $F^2\sigma_D^2$ is a component ascribable to special combining ability of lines in particular crosses. It can therefore be seen that until the level of inbreeding is well advanced, special combining ability will always play a subsidiary role to the general combining ability of the lines, unless the dominance variance is exceptionally large compared to the additive genetic component. Such a situation might occur if overdominant loci, with genes at intermediate frequencies, were contributing largely to the total variance. Employing a somewhat subjective assessment and applying the law of parsimony, it seems that overdominance at a number of loci was not encountered in this study.

The application of these results will be limited to situations of similar genetic control, but in conclusion, inbreeding and crossing as a method of improving a character such as the one described in this paper will not prove useful unless lines at a fairly advanced level of inbreeding are maintained. Even then, many if not most crosses may not be successful in increasing litter size. In view of this, it is encouraging that the within-family selection experiment, carried out on the same stock in this laboratory, has by now produced a substantial difference in litter size between the high and low lines (Falconer, 1955 and unpublished). It has just been shown that there is no reason to suppose that the character is controlled by many overdominant loci, which would preclude the successful outcome of a selection programme. Only in such circumstances would inbreeding and crossing be a better method of improving the character.

SUMMARY

1. The experiment was designed to provide basic information relevant to the utilization of heterosis in animal improvement. The character studied was the size of the first litter in mice.

2. Thirty inbred lines were crossed at random when the inbreeding coefficient reached 0·50 (three full-sib matings). The lines had been inbred without selection except for natural selection operating with lines.

3. The mean litter size of the crossbred mice did not exceed that of the outbred population from which the inbred lines had been derived. This indicates that the increased litter size normally associated with crossbred mice must be ascribed to some form of selection other than within-line natural selection.

4. Estimates were obtained of the variance components associated with general and special combining abilities. As anticipated, these estimates were very small, especially those relating to special combining ability. Before selection between crosses becomes possible, high levels of inbreeding must be achieved.

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New evidence of unbalanced sex-chromosome constitutions in the mouse

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INTRODUCTION

Russell, Russell & Gower (1959) and Welshons & Russell (1959) have shown that mice of the X/O sex-chromosome constitution can arise through failure to receive any sex chromosome (either an X or a Y) from the father. The X/O animals are fertile females. Assuming that the failure is due to the occurrence of non-disjunction during male gametogenesis, they estimate that the resulting O-sperms (lacking both X and Y chromosomes) must constitute about 0·6% of all sperms.

Welshons & Russell point out, however, that their extensive genetic tests failed to yield evidence for two predictions which can be made on the basis of these conclusions: (1) non-disjunction should also occur in the female, leading to the production of X/O females through fertilization of an egg lacking an X chromosome by an X-bearing sperm, and (2) X/X/Y animals should occur through fertilization of the egg by an XY sperm (the other possible product of male non-disjunction).

The above-mentioned work led me to re-examine data obtained in the course of investigating anomalous transmission of sex-linked factors in mice. As a result, not only was confirmation obtained of the phenomenon described by Russell, Russell & Gower and by Welshons & Russell, but also evidence came to light of the existence of the two missing categories listed above. In each case one example only was found, but, as will be seen, the rarity of the phenomena involved would make the collection of additional instances extremely arduous.

MATERIAL

Two stocks of mice were used. In the ancestry of stock A, the C57BL and C3H inbred strains, a multiple recessive stock, and a stock obtained from Prof. H. Grüneberg containing the sex-linked marker *Bent-tail* (*Bn*) (Garber, 1952) were represented in varying proportions, with C57BL predominating. Stock B was extracted from crosses between Stock A and a stock obtained from Dr D. S. Falconer containing the sex-linked marker *Tabby* (*Ta*) (Falconer, 1953).

RESULTS

1. *X/O females resulting from non-disjunction in the male parent*

One method of searching for females of the above type is to examine the daughters of a male carrying a totally sex-linked gene. Table 1 shows the offspring

of hemizygous *Bent-tail* males. Any daughter not carrying *Bn* is presumptively X/O. Unfortunately, with this marker the diagnosis cannot be made on phenotypic appearance alone since *Bn* is not fully penetrant in heterozygous females. Samples

Table 1. *Stock A matings to detect non-transmitters among the daughters of* Bent-tail *males. A female was classified as a non-transmitter if she gave no phenotypically* Bent-tail *mice out of more than twenty fully classified offspring. This criterion corresponded to a P value of less than 0·001*

Type of cross	No. of daughters	No. of wild-type daughters (non-manifesters plus non-transmitters)	No. tested	No. not transmitting *Bn*
Wild-type ♀♀ × *Bent-tail* ♂♂	373	58	36	1
Bent-tail ♀♀ × *Bent-tail* ♂♂	191	53	13	1

of phenotypically wild-type daughters were therefore test-mated in order to identify non-transmitters of the *Bn* gene.

It will be seen that 2 non-transmitters were identified out of 49 tested. The first gave 21 offspring, all wild-type, 9 males, 12 females, and the second gave 41 offspring, all wild-type, 15 males, 26 females.

An X^+/O female mated to a normal X^+/Y male is expected to produce four classes of young. Of these classes, Russell, Russell & Gower (1959) brought forward evidence to show that the Y/O type is lethal (half of all males die before birth), and the X/O type of female is inferior in viability to the X/X type (in their material only 60% as viable). The resulting sex-ratio among the young should be about 1·6 : 1. The sex-ratio among the offspring of the two non-transmitters (38 females, 24 males) agrees excellently with this expectation, but does not differ significantly from a 1 : 1 ratio.

Two further series of crosses, using stock B, were such as to reveal X/O females of similar origin, but failed to find any. In one, 746 daughters of *Tabby Bent-tail* males were raised, but all were phenotypically *Tabby* and hence must have received an X chromosome from their father. In the other series, *Tabby* females were mated to non-*Tabby* males. Any *Tabby* daughters failing to receive an X chromosome from their father should show hemizygous manifestation of the *Tabby* condition as demonstrated by Welshons & Russell (1959). Of 216 *Tabby* daughters, none did so.

The proportion of females which are X/O was calculated by Welshons & Russell (1959) to be approximately 1·2% in their material. For the present data, the upper line of Table 1 yields an estimate (allowing for the fact that only 36/58 normal daughters were test-mated) of 0·4% ($=\frac{1}{373}\times\frac{58}{36}$). The data in the lower line of Table 1 unfortunately cannot be used to improve the precision of this estimate, because the three frequency classes which it includes (*Bent-tail* females, non-*Bent-tail* transmitters, and non-*Bent-tail* non-transmitters) yield only two degrees of

freedom for purposes of estimation, and this is insufficient for estimating three unknown quantities, in this case (1) the viability of *Bn* homozygotes, (2) the penetrance in *Bn* heterozygotes, and (3) the frequency of O sperm (i.e. of sperm lacking both X and Y chromosome). The failure to find any X/O females of similar origin in the stock B crosses, out of a total of 962 relevant females, suggests that the estimate of 0·4% derived above may be too high.

2. *X/O female resulting from non-disjunction in the female parent*

Of the 746 daughters of *Tabby Bent-tail* fathers, all except one showed the typical female heterozygous manifestation of *Tabby*. The one exception, however, showed the more extreme phenotype characteristic of the hemizygous *Tabby* male or the homozygous female (there were no hairs, for instance, on the tail). The mother was an F_1 hybrid between two C3H substrains, and was of normal agouti phenotype. The exceptional animal had also inherited *Bn* from the father. Its tail was strongly but not acutely kinked, to a degree consistent with either heterozygous or hemizygous manifestation (although male hemizygous manifestation of *Bn* is much more marked than that of female heterozygotes, the two classes overlap). It appeared to have normal female external genitalia, with patent vagina, but died at the age of 10 weeks without having produced any young. This is not surprising in view of the low viability and fertility of *Tabby Bent-tail* males which it resembled in outward appearance.

The presumption is that the animal was an X/O female. Welshons & Russell (1959) report a total of six females with hemizygous *Tabby* manifestation which proved on analysis to be of X/O constitution. All six obtained their single X chromosome from the mother. The present female, on the other hand, must have obtained its X chromosome from the father, hence non-disjunction must have occurred in the *maternal* parent, leading to the production of an egg-cell with no X chromosome. No such patroclinous X/O female was observed by Welshons & Russell among 275 female offspring of appropriate crosses.

It could be argued that the anomalous female was an exceptional heterozygote showing full homozygous expression. Falconer (1953), in discussing variability of expression in *Tabby* heterozygotes, mentions that out of several hundred presumed heterozygous females, one showed the full phenotype characteristic of homozygotes. Since it failed to breed, its genotype could not be determined. Since then, two other similar cases have been observed (Falconer, personal communication), one involving *Brindled* (*Br*) and the other *jimpy* (*jp*). For these genes, the difference between heterozygous and hemizygous expression is so marked that the anomalous females cannot reasonably be regarded as being heterozygotes with extreme manifestation. In the light of this evidence, and also that of Welshons & Russell, it now seems more likely that Falconer's anomalous *Tabby* female was also an X/O hemizygote rather than a heterozygote with extreme manifestation. If so, it represents an additional case of non-disjunction in the maternal parent, since it is clear from Falconer's Table 1 (Falconer, 1953) that the

animal must have obtained its X chromosome from the father. In the appropriate cross, 1 out of a total of 48 *Tabby* females showed hemizygous expression.

On the other hand, the *Brindled* and *jimpy* X/O females must have obtained their sex-linked markers from the mother, since both genes are lethal in males; and must therefore have resulted from non-disjunction in the male parent, resembling in this respect the *Tabby* X/O females of Welshons & Russell.

3. *X/X/Y male resulting from non-disjunction in the male parent*

Non-disjunction in the male should result in the production of XY sperms as well as sperms lacking both X and Y. It is detectable by the transmission of a totally sex-linked gene from father to son.

Table 2 summarizes the relevant crosses which were made. The single anomalous mouse which appears in the table appeared on external examination to be a

Table 2. *Crosses in which the male parent carried a totally sex-linked gene or genes absent in the female parent. One case of father-to-son transmission was detected. The data given in the first line of the table derive from the same twenty-one matings as those in the first line of Table 1*

			Male progeny	
Stock	Type of cross	No. of matings	Receiving sex-linked gene(s) from father	Total
A	Wild-type ♀♀ × *Bent-tail* ♂♂	21	0	399
B	*Tabby* ♀♀ × *Bent-tail* ♂♂	28	0	516
B	Wild-type ♀♀ × *Tabby Bent-tail* ♂♂	42	1	747
	Total	91	1	1662

male, but the manifestation of both *Ta* and *Bn* was of the heterozygous female type. For *Bn* the distinction between hemizygous and heterozygous expression is not absolute, since, as explained above, the hemizygous and heterozygous conditions constitute a continuous though bimodal distribution; but with *Ta* the hemizygous expression is extremely constant and distinctive, and no possibility of misclassification arises in the male. The presence of hairs on the tail, for instance, has never been reported in a hemizygous male. The animal was stunted in growth and died at the age of 25 days. Its sex was checked by post-mortem dissection. No macroscopic signs of intersexuality were observed. The mother was a C3H × C57BL F_1 hybrid.

On the hypothesis of non-disjunction we must presume this male to have been X/X/Y in chromosomal constitution, the result of fertilization of a normal egg-cell containing one X chromosome by a sperm bearing both an X and a Y chromosome. Not only is this conclusion indicated by the mode of inheritance of the sex-linked markers, but also by the highly anomalous expression of the *Tabby* gene. The estimate of 1/1662 for the frequency of X/X/Y males obtained from Table 2 is an

underestimate, since, from what is known of *Bn* manifestation in female heterozygotes, not all the $X/X^{Bn}/Y$ males produced by the first two types of cross would be expected to show the *Bent-tail* phenotype.

DISCUSSION

The anomalous observations described above were of three distinct types. The chief merits of the hypothesis of unbalanced sex-chromosome constitution, arising presumably as a result of non-disjunction, are (1) that it enables them all to be accounted for under a single theory, and (2) that it is rendered *a priori* likely as a result of the work of Welshons & Russell (1959). Their combined genetical and cytological analysis has established that females of X/O chromosome constitution which have received their single X chromosome from the mother may occur with a frequency as high as 1%: if the very considerable number of mice bearing sex-linked markers which was raised in the course of the work described in the present paper had *not* yielded some similar animals, the discrepancy would itself have required explanation. The other two types of unbalanced sex-chromosome constitution implicated by the present work, namely X/X/Y animals, and X/O females which have received their single X chromosome from the father, might both *a priori* be expected to arise as a result of the same type of failure of orderly meiotic distribution of the sex chromosomes as is responsible for the production of X/O females receiving their single X chromosome from the mother. The single animal of each type which came to light can therefore be considered as further confirmatory evidence for the phenomenon uncovered by Russell, Russell & Gower (1959) and Welshons & Russell (1959).

Similar unbalanced sex-chromosome constitutions have recently been reported in the human species. Ford, Jones, Polani, de Almeida & Briggs (1959) report a case of Turner's syndrome (a condition of women, in which the gonads are absent or rudimentary, other congenital malformations are present, and characteristically the female sex chromatin is absent from the skin cells) where on the basis of chromosome counts it was concluded that the sex-chromosome constitution was X/O. In this case there was no way of determining which parent had transmitted the single X chromosome; but in four other cases of chromatin-negative Turner's syndrome, studies of the inheritance of sex-linked recessive colour-blindness suggested that the non-disjunctional event leading to the production of a gamete lacking any sex chromosome occurred in the male parent (for references see Ford, Jones, Polani, de Almeida & Briggs, 1959). Evidence has also been presented (Jacobs & Strong, 1959; Ford, Jones, Miller, Mittwoch, Penrose, Ridler & Shapiro, 1959) that two cases of Klinefelter's syndrome (male-type intersexes, with under-developed testes) showing female sex chromatin in the skin cells were X/X/Y in chromosome constitution. The three cases of colour-blind Klinefelter individuals which have been detected so far (Nowakowski, Lenz & Parada, 1959) apparently arose through fertilization of an XX ovum by a normal Y sperm (Stern, 1959), rather than through fertilization of a normal ovum by an XY

sperm, as in the presumptive case of an X/X/Y mouse described in the present paper.

A single case has been reported of a human female with forty-seven chromosomes, apparently X/X/X in chromosomal constitution (Jacobs, Baikie, Court Brown, MacGregor, Maclean and Harnden, 1959). In *Drosophila* such 'super females' are often inviable; but in Man only the genital tract seems to be affected. A proportion of the buccal mucosa cells contained two sex-chromatin bodies, rather than the normal female complement of one. The term 'super female' is accurate only in respect of chromatin, since the genitalia were under-developed, and the woman almost certainly sterile.

It is interesting to note that in humans the 'female' sex chromatin appears to be associated with the possession of two or more X chromosomes, since it is present in X/X females and X/X/Y males, present in larger quantity in the X/X/X 'super female', but absent in X/O females and X/Y males. The gross morphological characteristics of maleness and femaleness seem, however, to depend on the presence or absence of the Y chromosome, both in the human species and in the mouse.

Welshons & Russell (1959) discuss whether in the mouse the X/X/Y constitution would be expected to lead to intersexual or to fully male development. The single presumed X/X/Y animal reported in the present paper appeared to be fully male, but it did not survive long enough to enable its fertility to be tested.

The phenotypes now believed to correspond, in the mouse and Man respectively, to the unbalanced sex-chromosome constitutions discussed above are summarized in Table 3; while Table 4 lists the various non-disjunctional mechanisms by which these constitutions may arise, assuming non-disjunction in one parent only.

Table 3. *Phenotypes corresponding to anomalous sex-chromosome constitutions*

	Species	Phenotype	Reference
X/O	Mouse	Fertile female	Russell, Russell & Gower (1959); Welshons & Russell (1959); Present paper
	Man	Sterile female; Chromatin negative ('male'); (Turner's syndrome)	Ford, Jones, Polani, de Almeida & Briggs (1959)
X/X/X	Mouse	Not known	—
	Man	Female, genital tract underdeveloped; Chromatin positive, some cells with two chromatin bodies ('super female')	Jacobs, Baikie *et al.* (1959)
Y/O	Mouse	Probably inviable	Russell, Russell & Gower (1959)
	Man	Not known	—
X/X/Y	Mouse	Male, fertility not known	Present paper
	Man	Sterile male; Chromatin positive ('female'); (Klinefelter's syndrome)	Jacobs & Strong (1959)

If X/X/Y mice are indeed viable males or near-males, why do they apparently occur so rarely compared with X/O females? Welshons & Russell (1959) presume that their eight matroclinous X/O females arose through failure of the paternal XY bivalent to disjoin at meiosis, resulting in sperm which carried neither an X nor a Y chromosome. They do not offer any explanation of their failure to detect the complementary non-disjunctive type, that is the X/X/Y animals which should result from fertilization by sperm carrying both an X and a Y chromosome,

Table 4. *Modes of origin of anomalous sex chromosome constitutions*

(Chromosomal constitutions of gametes produced as a result of non-disjunction are printed in bold type)

Type	Sperm	Egg	Genotype	Species	Reference
Non-disjunction in female parent	X	**O**	X/O	Mouse	Present paper
					Falconer (1953)
				Man	Not reported
		XX	X/X/X	Mouse	Not reported
				Man	Jacobs, Baikie *et al.* (1959)
	Y	**O**	Y/O	Mouse	Russell, Russell & Gower (1959)
				Man	Not reported
		XX	X/X/Y	Mouse	Not reported
				Man	Stern (1959)
Non-disjunction in male parent*	**O**	X	X/O	Mouse	Russell, Russell & Gower (1959)
					Welshons & Russell (1959)
					Present paper
				Man	Ford, Jones, Polani, de Almeida & Briggs (1959)
	XY	X	X/X/Y	Mouse	Present paper
				Man	Not reported

* Non-disjunction during the second meiotic division could in theory lead to the production of XX and YY sperm also, and hence to X/X/X and X/Y/Y animals.

though these might in theory be expected to occur as frequently as the X/O types. However, in *Drosophila*, where non-disjunction of the X chromosomes has been intensively studied, the frequency of XX eggs is about 1 in 2500, while the frequency of no-X eggs is 1 in 600, or more than four times as high. To quote Sturtevant & Beadle (1939): 'This difference is evidently due to the fact that failure of one of the X chromosomes to be included in a daughter nucleus at the first division is a more frequent deviation from normal than is the inclusion of both X's in a single daughter nucleus.' A corresponding asymmetry in the assortment of the paternal X and Y chromosomes in mammals would account for the higher incidence of the X/O than the X/X/Y chromosome constitution.

SUMMARY

Some anomalous results are described of genetical tests using the sex-linked markers *Tabby* and *Bent-tail*. The results are shown to be consistent with the demonstration by Welshons & Russell (1959) that the X/O chromosome constitution is female, and suggest that the condition can arise from non-disjunction in the female as well as from non-disjunction in the male parent. A single animal of presumed X/X/Y constitution appeared anatomically to be fully male, but died before breeding tests could be performed.

Note added in proof

In writing this paper, it was assumed that the non-disjunctional event leading to the production of unbalanced sex-chromosome constitutions occurred in the parent, during the course of gametogenesis. Genetically identical results would be observed if non-disjunction of the sister chromatids of one of the sex chromosomes took place in the first cleavage division of the zygote, provided that the other aneuploid blastomere either died or failed to contribute to the further development of the relevant parts of the embryo. Ohno, Kaplan & Kinosita (1959) have subsequently reported that cytological examination of 2192 meiosis figures from fifteen male mice belonging to six inbred strains 'failed to reveal a single instance of mishap which might lead to the formation of XY- and O-sperm'. The discrepancy between this result and Welshons & Russell's estimate of 0·6% O-sperm is suggestive evidence that most or all of the non-disjunction occurs at the first cleavage division rather than during gametogenesis. But it is not conclusive, since the frequency of non-disjunction might vary widely between different stocks of mice, between different individuals, or even in the same individual at different ages.

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Inbreeding depression and heterosis of litter size in mice

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1. INTRODUCTION

The objects of the experiment described in this paper were the following:

1. To measure the rate of decline of litter size with inbreeding.
2. To see if the rate of decline was influenced by selection within lines.
3. To see what improvement in litter size could be made by crossing the best inbred lines.

The utilization of heterosis depends on selection applied either during the inbreeding or at the crossing of the inbred lines. In another experiment carried out with the same strain of mice, Roberts (1960) showed that the crossing of lines inbred without selection yielded no improvement in litter size; and that lines crossed at 50% inbreeding varied little in either general or special combining ability, so that selection applied to the crosses gave little promise of improvement. The experiment to be described here explores the possibility of improvement by selection applied to the lines during the inbreeding, first by selection of the best individuals within each line, and second by selection of the best lines judged on their average performance as inbreds.

Selection without inbreeding was applied in another experiment which ran concurrently with the inbreeding experiment described here. The results, of which a preliminary report was given by Falconer (1955), will be described in another paper.

2. PROCEDURE

History of stock used

The strain of mice used for all the experiments on litter size, known in the laboratory as the J-strain, was a heterogeneous one of mixed origin. In its past history there had been little inbreeding that might have eliminated deleterious genes, and it was about as close as one can get with laboratory mice to a 'natural' random-bred population. It had been made from crosses between Bateman's high-lactation line, Goodale's and MacArthur's large selected lines, and four mutant stocks with the C57BL inbred strain in parts of their ancestries. The following mutant genes were originally present: *a*, a^t, *b*, *bt*, *c*, c^{ch}, *Ca*, *d se*, *ln*, *m*, *Re*, *si*. This stock was first used in another experiment (Falconer & Robertson, 1956), which however, resulted in no detectable genetic changes. The 'experimental' and

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'control' lines from that experiment were crossed after each had been maintained through ten generations by eight pairs of parents per generation with minimal inbreeding. Crossbred progeny drawn equally from twenty different matings were used to form the base populations for the inbreeding experiment here described and for the selection without inbreeding. This latter base population was continued without selection to act as a control for both experiments. It was designated JC and was maintained by ten single-pair matings per generation with minimal inbreeding, so that the effective population size was forty and the theoretical rate of inbreeding was 1·25% per generation.

Measurement of litter size

The mice were mated at the age of 6–9 weeks in harems of up to five females with one male. The females were isolated 18 days after mating and were subsequently examined daily for litters. The measure of litter size adopted was the number of live young present when the litter was found. Only first litters were recorded. Females that failed to produce any live young, even though previously noted as being pregnant, were excluded from all estimations of the mean litter size. The inclusion of still-born young or of zero-litters would have introduced an undesirable element of chance variation into the measure of mean litter size because the recording of still-born young depends on the mother not having eaten them before the litter is found, and because many abortive pregnancies would go unnoticed. Mean litter sizes therefore refer only to those females that produced at least one live young within about 6 weeks of mating.

Litter size as a 'character' for genetic analysis is complicated by having two components, one attributable to the fertility of the mother of the litter and the other attributable to the prenatal viability of the young in the litter. Furthermore, it is subject to an inverse maternal effect: mothers who were themselves reared in a large litter tend to have small litters because their body size is smaller (Falconer, 1955). For these reasons we shall not attempt to go much beyond a simple description of the results of this experiment. Further procedural details will be given in association with the results to which they are relevant. Inbreeding coefficients throughout the experiment were computed from the actual pedigrees, by the method of Cruden (1949).

3. INBREEDING PROGRAMME

Method of inbreeding

The inbreeding was started by matings between double first cousins, and continued thereafter by full-sib matings. The purpose of starting by cousin matings was to put in an intermediate step at 12·5% inbreeding, instead of jumping immediately to 25% as with full-sib matings. The inbreeding coefficients in the successive generations are given in Table 1. The matings between the mice of the base population were arranged so as to produce non-inbred progeny in families of which pairs were related as double first cousins. There were ten such pairs of families, and matings between them constituted the first inbreeding and divided the population into ten lines of

independent ancestry. The non-inbred mothers with 12·5% inbred litters were designated generation O.

Throughout the inbreeding programme each line was propagated from a single litter of the previous generation. All the females of the litter were mated to the same male, or if there were more than five females they were mated in two harems to two males of the same litter. The litters born of these matings provided the measure of the litter size of that line in that generation. One litter was reared for the continuation of the line and the rest were discarded. Propagation, and the measurement of litter size, were exclusively by first litters. Lines became extinct when no litter containing one surviving offspring of each sex was available.

Method of selection

At generation O each of the ten lines was divided into two, one to form the unselected series of lines, designated JU, and the other to form the selected series, JS. Thus each line of the selected series was related to one of the unselected series. Each line of the selected series was propagated always from the largest of the litters born in the previous generation of that line. The litters from which the unselected series of lines were propagated were not taken strictly at random, but were rather those of intermediate size and containing the highest proportion of females. This was done in order to reduce the chance effects of random sampling, and to provide as many litters in the next generation as was possible without any positive selection. The comparison of the selected with the unselected series of lines provided the test of the efficacy of artificial selection applied within the lines. Selection between lines was entirely 'natural': lines of both series became extinct, as already explained, as soon as they failed to produce a sib-pair for their propagation.

Results

The results of the first twelve generations of inbreeding are given in Table 1, and the first four generations are shown graphically in Fig. 1. The mean litter sizes given in Table 1 are the means of all litters irrespective of their line; but those plotted in Fig. 1 are the unweighted means of line-means. The means of the selected series were a little above those of the unselected series, but the difference is quite insignificant, and the selection quite failed to delay the loss of lines; indeed, the lines became extinct sooner than those in the unselected series. The selection differentials, given in Table 1, were however rather small, the selected litters exceeding the average by only about $1\frac{1}{2}$ mice. This amount of selection, though it was the most that could be achieved with the procedure adopted, could hardly be expected to show any effect. The failure of selection within lines to reduce the rate of decline is therefore to be attributed to the low intensity of selection and is not necessarily proof that the selection itself was ineffective.

The decline of litter size depicted in Fig. 1 is very regular and is linear with respect to the inbreeding coefficient. The rate of decline is 0·56 young per 10% increase of inbreeding. The comparable figure found by Roberts (1960) was 0·49 young. If the

Table 1. *Inbreeding coefficients* (F) *and mean litter sizes in the first twelve generations of inbreeding. The means are of all litters, irrespective of lines*

		Unselected Lines					Selected Lines					
					Litter size					Litter size		
Generation	*F* (%) of litters	No. of lines surviving	No. of ♀♀ mated	No. of litters	Mean	s.e.	No. of lines surviving	No. of ♀♀ mated	No. of litters	Mean	s.e.	Selection differential
Base	0	—	30	30	7·77	0·48	—	—	—	—	—	—
0	12·5	10	45	43	6·88	0·43	—	—	—	—	—	1·88
1	31·3	10	29	28	6·32	0·55	10	37	33	5·58	0·46	1·73
2	43·8	10	29	27	5·04	0·51	10	34	34	5·68	0·41	1·63
3	54·7	9	19	18	5·00	0·57	10	35	28	5·32	0·46	1·75
4	63·3	6	16	12	4·58	0·60	4	17	8	4·38	0·60	1·00
5	70·3	4	11	8	5·89	0·99	1	1	0	—	—	—
6	76·0	3	10	8	6·63	0·80	0	—	—	—	—	—
7	80·6	3	19	14	7·71	0·54						
8	84·3	3	16	13	6·08	0·45						
9	87·3	3	23	19	5·21	0·56						
10	89·7	3	27	21	7·29	0·54						
11	91·7	2	28	11	5·18	0·77						
12	93·5	1	20	19	7·58	0·60						

linear decline in the present experiment had continued to higher levels of inbreeding, the litter size would have been reduced to two at 100% inbreeding. After generation 4, however, the mean litter size increased because the worst lines had become extinct.

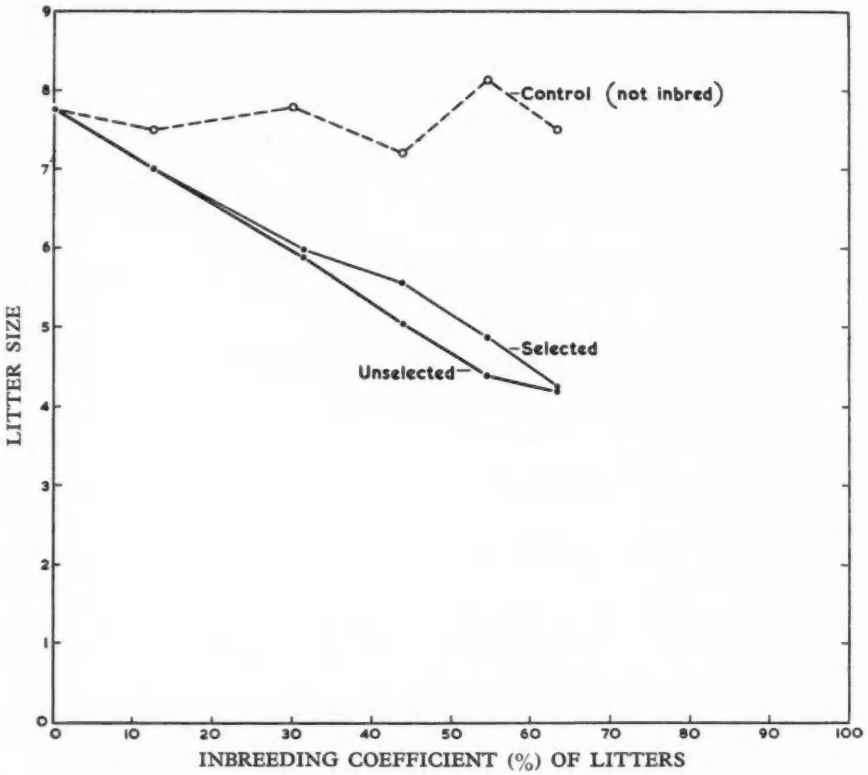

Fig. 1. Mean litter size plotted against the inbreeding coefficient of the litters. The points are the means of line-means.

Out of the whole twenty lines, one became extinct in generation 3 and all but one were extinct by generation 12. The distribution of losses was as follows:

Generation No.	2	3	4	5	6 ... 10	11	12
Total no. of lines extinct	0	1	10	15	17	18	19

Most of the lines were lost at generations 4–5, i.e. when the inbreeding coefficient reached 60–70%. Three lines, however, survived to 90% inbreeding and one survived permanently. It is of interest to inquire into the individual histories of these three longest-surviving lines: did they start at high levels and decline like the others? Or, did they suffer less decline than the others? The individual histories, shown in Fig. 2, prove the latter: these three lines started below the average and suffered no decline up to about 80% inbreeding at generation 7 or 8. Then two of

them dropped and were lost at generations 10 and 11. The remaining line, which survived permanently, is shown in Fig. 2 up to generation 20, when the inbreeding coefficient was 98·8%, and its mean litter size was then fully equal to that of the non-inbred control.

In preparation for the crossing programme to be described below, the three surviving lines were expanded at generation 6 by the use of two litters for their continuation instead of one. All the litters of generation 7 were used for the crossing or the continuation of the lines, and thereafter all available litters (except unisexual ones) were used for the continuation. This expansion did not, however, prevent the

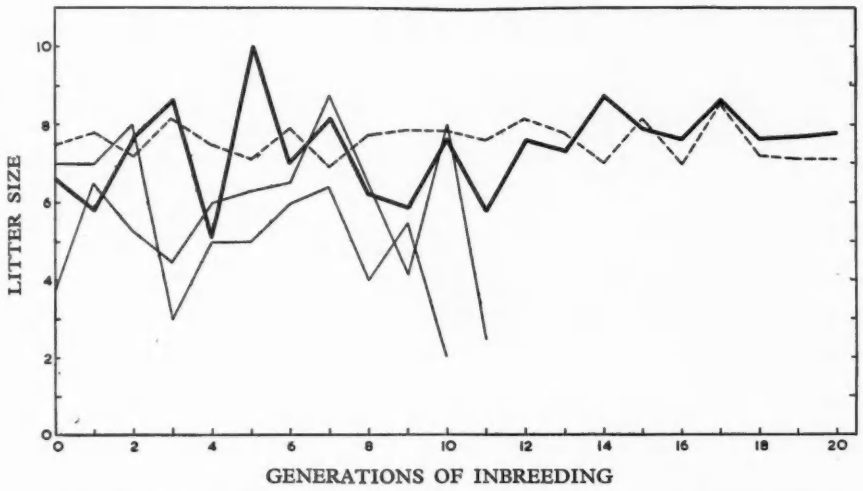

Fig. 2. Individual histories of the three lines that survived longest. The dotted line refers to the unselected, non-inbred control. The line means in the early generations are based on very few litters. The means of the permanently surviving line are based on about twenty litters in generations 12–16 and on about forty in generations 17–20.

loss of two of the lines, but the permanent survival of the remaining line was probably helped by the selection between sublines which was applied from generation 12 onwards, when the maintenance of the line became a matter of stock-keeping rather than of experiment.

4. CROSSING PROGRAMME

The crossing programme consisted of crosses between the best inbred lines followed by renewed inbreeding and crossing. Three such cycles of inbreeding followed by crossing were carried out. The procedure will be most easily understood if described step by step along with the results.

The results are given in Table 2 and the mean litter sizes are shown graphically in Fig. 3. This figure is divided into sections corresponding to the successive cycles of inbreeding and crossing. The horizontal axis in each section is marked out in

Table 2. *Inbreeding coefficients and mean litter sizes in the crossing programme*

Generation*	Inbreeding coefficient (%)		No. of litters	Litter size	
	Mothers	Litters		Mean	S.E.
8	80·6	84·3	13	6·08	0·446
X_1	80·6	0	17	7·53	0·550
0	0	20·0	40	9·58	0·365
1	20·0	35·0	93	8·33	0·278
2	35·0	47·5	75	7·28	0·308
3	47·5	57·5	68	6·90	0·325
4	57·5	65·6	47	6·89	0·363
X_2	65·6	25·7	59	7·80	0·315
0	25·7	26·5	57	7·90	0·283
1	26·5	37·8	54	7·15	0·268
2	37·8	50·8	68	8·47	0·233
3	50·8	59·7	54	7·37	0·324
4	59·7	67·7	35	6·00	0·467
X_3	67·7	38·2	40	6·83	0·308
0	38·2	38·3	106	7·32	0·210

* Explanation of generation numbers:

X_1, X_2, X_3: crosses between lines after first, second and third cycles of inbreeding.
0: second generations of crosses, with minimal inbreeding of mothers and litters.
1, 2, 3, 4: first, second, etc. generations of inbreeding.

coefficients of inbreeding, and the generation means are plotted against the coefficient of inbreeding of the litters. The coefficient of inbreeding of the mothers is thus represented throughout by the previous point in the graph. The left-hand

Fig. 3. Mean litter size in successive generations of the crossing programme, plotted against the coefficient of inbreeding of the litters. The horizontal broken line is the mean of the non-inbred control over the same period.

section of the figure represents the first cycle of inbreeding described in the preceding section. The litter sizes plotted here are the means of all litters in both the selected and unselected series. The litter size declined regularly, as we have already seen, up to 63% inbreeding. Then it rose again steeply as the worst lines became extinct till, at 81% inbreeding when only three lines remained, it was equal to the non-inbred control. At this point the three lines were crossed in all ways including reciprocals. The mothers of these crossbred litters were 81% inbred, and the litters themselves were zero-inbred. The point representing the cross on the graph is therefore shown against 0% inbreeding in the second cycle. There was no improvement of litter size. The crossbred progeny were then mated with others from a different cross, so as to produce litters with minimal inbreeding. The inbreeding coefficient of these litters did not, however, remain at zero, but increased to 20% because with only three lines in the cross, the mated pairs of F_1's had always one parental line in common. The next point on the graph, then, refers to 20% inbred litters with 0% inbred mothers. There was now a marked improvement, the mean litter size increasing to 9·6. Thus inbreeding followed by crossing the best lines led to an improvement of two mice per litter in a space of ten generations. Selection without inbreeding, as will be shown in another paper, took twice as long to achieve the same improvement.

The next step was to subject the crossbred population to a second cycle of inbreeding and crossing to see if this would lead to further improvement. Twenty litters were kept, and inbreeding by full-sib mating was ntinued for four generations. The mean litter size among these twenty lines declined at almost exactly the same rate as in the first cycle of inbreeding. But since the lines started at a higher level they did not fall so low, and only three out of the twenty were lost. After four generations, when the litters were 66% inbred, the four best lines were selected on the basis of their performance as inbreds, and these four lines were crossed. The inbreeding coefficient of the crossbred litters was not zero as before, but 26%. This was the result of the initial restriction of the population to three lines in the first cross. The F_1 litters were again crossed to members of different F_1's so that both mothers and litters would be minimally inbred; and, because this time there were four instead of three lines, the inbreeding coefficient of the litters was very little more than that of the mothers. The mean litter sizes of these crosses are shown by the first two points, close together, at about 25% inbreeding in the third cycle. It will be seen that there was some improvement from the heterosis in the litters, but no more in the next generation from the heterosis in the mothers. This second cycle of inbreeding and crossing brought the litter size to a little above the control level, but the level attained by the first cycle was not regained.

The third cycle of inbreeding was carried out in the same manner as the second, except that the first generation was not sib-mated. Matings with least relationship were made, with the intention of producing a second minimally inbred generation, but because of the previous restriction to four lines, the inbreeding coefficient increased from 26·5% to 37·8%. Three generations of full-sib mating followed. The mean litter sizes in the third cycle were rather erratic, but the average decline was at about the same rate as before, and by the time the litters were 68% inbred the

mean litter size had dropped to six. The four best lines were again selected and crossed. This time, because of previous restrictions of the population size, the inbreeding coefficient of the crossbred litters was nearly 40%. There was again some improvement from the heterosis in the litters. The crossbred mice were mated to others from a different cross and some further improvement in litter size was realized (this is the last point shown on the graph), but, even so, the litter size was still below the control level. The experiment was terminated at this point.

As a means for the improvement of litter size, the second and third cycles of inbreeding and crossing were conspicuously unsuccessful. There are two reasons for this. First, the restriction of the population to three lines in the first cycle and to four in the second and third cycles made it impossible to return to zero inbreeding in the second and third crosses, so that the full potentialities of heterosis could not be realized. The second reason, which has not yet been mentioned, concerns the selection between the lines. In the first cycle natural selection had eliminated all but three of the lines and there was no doubt that these were the best lines and that they were substantially above the others in performance. In the second and third cycles, however, where the selection was artificial and not natural, it was difficult to decide which were the best lines. The line means, generation by generation, were very irregular, and no lines stood out as being very clearly superior to the rest. For this reason the efficacy of the selection, on which any improvement must rest, is open to question, and the results indicate its ineffectiveness. If the graph is examined it will be seen that the mean litter size at corresponding levels of inbreeding is the same in the second and third cycles and the third cross which represents the beginning of a fourth cycle of inbreeding. For example, the litter size is about equal to the control level at about 40% inbreeding in the three cycles. Thus it appears that selection was effective only in the first cycle and that the improvement gained could not be maintained because the low inbreeding coefficient of the first cross could not be repeated.

5. COMPONENTS OF VARIANCE

The variance of litter size was analysed into two components, within and between groups of full-sister females. It will be remembered that during the inbreeding all the females of one litter were mated to one male (or occasionally to two males) and that this group of females represented the whole of one line in one generation. The analyses of variance were made on the sizes of litters produced by these groups of females. In the inbred generations the within-group component refers to the variance within lines, and the between-group component to the variance between lines. But the between-line variance is not purely genetic because it contains also the variance arising from maternal effects since all the litters in a line were produced by mothers who were litter-mates. In the crossbred generations the components are difficult to interpret because of the dual nature of the character, and there would be little gained from a detailed discussion of their content. A more meaningful analysis of the crossbred generations will be presented later.

The estimates of the two components of variance throughout the experiment are

depicted in Fig. 4. The within-group component remained substantially the same and was not influenced in a regular manner either by the inbreeding or by the crossing. The between-group component apparently started at a level considerably above the control and it declined during the first cycle of inbreeding. During the second and third cycles of inbreeding it may have increased a little but it did not rise much above the control level. The small amount of variance differentiating the lines accounts for the difficulty found in applying selection, which was mentioned at the end of the last section.

Neither of the components of variance described above behaved altogether as they might have been expected to. The genetic variance within lines might be expected to increase during the first stages of inbreeding from the effects of rare recessive genes (Robertson, 1952), but it should decline later. The environmental

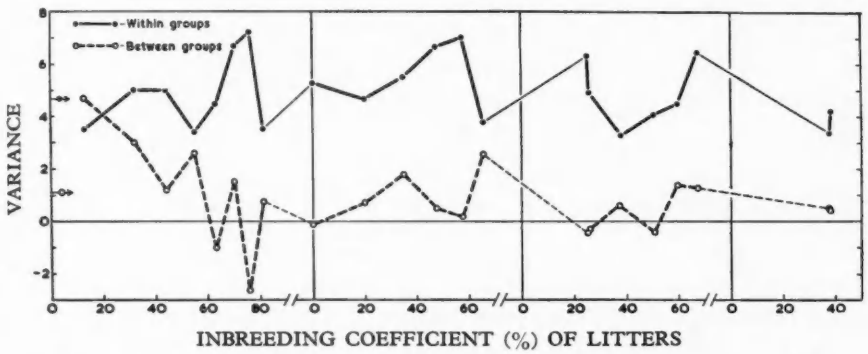

Fig. 4. Components of variance of litter size within and between groups of full sisters. The arrows on the left mark the components in the non-inbred control over the same period.

variance within lines might be expected to increase with inbreeding. The approximate constancy of the observed within-line variance may have been the resultant of these two more or less opposing underlying changes. The variance between lines should on theoretical grounds have increased with inbreeding. Its observed decline in the first cycle may have been due in part to a spuriously high initial level, but was probably due more to the elimination of the lines as inbreeding proceeded. The reliability of the estimates does not justify further comment on the changes of variance. It might, however, be pointed out that the sampling errors of the estimates of the two components are negatively correlated. If the within-group component is erroneously high, the between-group component will appear correspondingly too low. This accounts for much of the irregularity of the between-group estimates.

The variances of the crossbred generations were analysed in a different way, in order to see what evidence there was of general or specific combining ability of the lines crossed. The first cross was not analysed because the numbers were too small. The mean squares from the analyses of the second and third crosses are given in Table 3. The analyses separate three mean squares in addition to the error mean

square. The first and second, attributable to lines used as female parents and lines used as male parents, refer to general combining ability, and the third, attributable to interaction between lines, refers to special combining ability. The results are somewhat ambiguous. Each of the three mean squares referring to combining

Table 3. *Analyses of variance of the crosses*

	Parents inbred, litters crossbred		Parents crossbred, litters crossbred	
	d.f.	Mean square	d.f.	Mean square
2nd cycle				
♀ lines	3	12·45*	3	2·80
♂ lines	3	5·06	3	4·84
Interaction	5	5·68	5	10·44†
Error	47	5·50	45	4·01
3rd cycle				
♀ lines	3	6·93	3	2·40
♂ lines	3	12·26†	3	7·67
Interaction	5	0	5	3·00
Error	27	3·58	94	4·74

* $P < 0{\cdot}10$. † $P < 0{\cdot}05$

ability is significant in one of the four analyses but not in the others. These results give general support to the conclusions of Roberts (1960) in showing little evidence of differences in combining ability between the inbred lines.

6. CONCLUSIONS

There are two chief points of interest in the conclusions that can be drawn from this experiment. The first concerns the genetic situation underlying the inbreeding depression and heterosis of litter size, and the second concerns the utilization of heterosis in farm animals.

The decline of the mean value on inbreeding proves that there must be directional dominance of the loci concerned, but it does not discriminate between simple dominance and over-dominance. The behaviour of the individual lines, however, proves that in this case over-dominance cannot have been of paramount importance. If much of the original variation had been due to over-dominant loci it would hardly have been possible to find three lines that showed no inbreeding depression up to 80% inbreeding, and one that showed none up to 99% inbreeding. The behaviour of these lines is adequately accounted for by the hypothesis of simple dominance. Favourable dominant alleles, one may suppose, were fixed, or brought to high frequencies, at many loci and unfavourable recessives at a few loci. On balance there was no change of mean, but there was heterosis on crossing because the recessive alleles in each line were different. The decline of the mean observed in the second cycle of inbreeding must then be attributed principally to these loci that gave rise to the heterosis. That the decline was not slower than that of the first cycle is

understandable because the gene frequencies at these loci must all have been about one-third, whereas in the original base population the gene frequencies may well have been much lower, and the contribution of any locus to the inbreeding depression is dependent on its gene frequency.

The conclusions about the utilization of heterosis by cyclical inbreeding and crossing are not encouraging, if the experiment is regarded as an indication of what may be expected to happen with farm animals. The similarity between litter size in pigs and litter size in mice is probably very close, both genetically and physiologically, and the experiment seems to be very relevant to the improvement of productivity in pigs. Selection within lines during the inbreeding does not seem likely to be able to reduce the rate of decline of performance. This is in agreement with previous data from pigs, reported by Dickerson (1952). On the other hand, selection between lines on the basis of their performance as inbreds does encourage the hope that some useful heterosis may be gained by the crossing of the best lines. Since, however, crosses between the less-good inbreds were not tested we cannot exclude the possibility that these might have yielded better hybrids than the crosses between the best inbreds, as was found by Bell, Moore & Warren (1955) with egg-laying in *Drosophila*. With farm animals the maintenance of poor inbreds is impracticable, and if the lines are to be perpetuated for the continuous production of crossbreds, the selection would have to be made out of a very large number of lines if some are to be found that retain a high enough performance as inbreds for their maintenance to be practicable. There does not seem to be much hope of making further gains from the cyclical repetition of inbreeding and crossing, because of the difficulty of returning to a low level of inbreeding in the second and subsequent crosses. Each selection among the lines for crossing reduces the population size and so puts the population through a 'bottle-neck' from which it can never subsequently recover. Thus if the full amount of heterosis is to be achieved in a later cross, all the previous crosses would have to be made among a large number of selected lines. Success in a programme of cyclical inbreeding and crossing would require operations on a very large scale.

SUMMARY

1. A random bred population of mice was subjected to inbreeding and the changes of litter size, measured as the number of live young in first litters, were followed.

2. The mean litter size declined at a rate of 0·56 young per 10% increase of the inbreeding coefficient.

3. Selection for large litters within the lines during the inbreeding did not effectively reduce the rate of decline.

4. Out of twenty lines at the beginning of the inbreeding seventeen were lost by the time the inbreeding coefficient reached 76%. Two more were lost later and one survived indefinitely. The three lines that survived longest started at a level below the mean and did not decline in litter size. The one that survived indefinitely reached 99% inbreeding without dropping below the non-inbred control.

5. The three lines surviving at 81% inbreeding were crossed and the litters produced by the crossbred progeny were larger than the non-inbred control by about two young per litter. This gain from heterosis is attributable to selection among the lines on their performance as inbreds. A second and third cycle of inbreeding and crossing yielded no further progress, and the level of the first cross was never regained. This is attributable to the ineffectiveness of the selection applied and to the previous restrictions of the population size.

6. The behaviour of the lines in the inbreeding and crossing point to simple dominance rather than over-dominance at the loci causing variation of litter size.

7. This experiment suggests that, as a means of improvement of farm animals, cyclical inbreeding and crossing does not look very hopeful.

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Four new half-and-half mosaic fowls

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(*Received* 28 *October* 1959)

INTRODUCTION

Half-and-half mosaics, i.e. genetic chimaeras whose two components comprise, at least approximately, the right and left halves of the body, have been recorded in several species of birds (references in Hollander, 1944). In mammals, on the other hand, they seem to be almost unknown; all the mosaics mentioned by Robinson (1957) are pigmentation mosaics with irregular and relatively small abnormal patches, although Zlotnikoff (1945) has described a human mosaic with a half-and-half arrangement, and it is possible that some of the cases of lateral hermaphroditism in rodents are mosaics (see Glass, 1945).

In the domestic fowl, at least twenty-two half-and-half cases have been recorded (Blyth & Hale, 1953; Jerome & Huntsman, 1959; other references in Cock, 1955); there are besides, a few cases which are probably half-and-half, but where a more restricted type of mosaicism cannot be excluded. In most cases the two sides of the body have differed in size, as well as in one or more such characters as skin colour, plumage pigmentation and structure, and eight of them have had hermaphroditic gonads (right testis, left ovary or ovotestis). Their mode of origin has been a long-standing puzzle. Loss or non-disjunction of a single chromosome at first cleavage has been the favoured explanation for most of them, but it is not universally applicable. Each of the three cases of Greenwood & Blyth (1951) and Blyth (1954) is mosaic for a sex-linked and an autosomal gene; there are also serious, if less obviously decisive, obstacles to applying a single-chromosome explanation to several other cases (Cock, 1955). Alternative explanations which have been proposed for some cases include the involvement of polar bodies (fertilized or unfertilized), or of supernumerary spermatozoa (giving rise to tissues of purely paternal origin), and the maldistribution of two or more chromosomes at first cleavage. The inevitable uncertainties due to incomplete information (e.g. uncertain parentage, lack of suitable marker genes in the parents, absence of a progeny test) are increased by the fact that no one explanation is capable of accounting for all the known cases.

Two of the cases now to be described are of particular interest, since, like those of Greenwood & Blyth, they are mosaic for both sex-linked and autosomal genes. They are, moreover, full brothers, and family information about them is unusually complete; parents, normal siblings and offspring have all been examined. The other two cases are less remarkable and will be described more briefly. All four cases are cross-bred, and all have the Light Sussex as one parental breed, the other breeds involved

being Brown Leghorn, Indian Game (Dark Cornish) and Rhode Island Red. Some preliminary account of the genetics of the breed differences is necessary; the important differences are summarized in Table 1.

The genes affecting skin colour and comb (*W* and *P*) are dominants which segregate sharply, but the genetics of the plumage colours and patterns is rather more complex. The black-red plumage of the Brown Leghorn, with its strong sexual dimorphism, may, following Hutt (1949), be taken as a convenient 'wild' or standard type. The plumage of the Light Sussex differs from this in two respects: (i) black pigment is restricted to parts of the wings, neck and tail (the so-called Columbian pattern); (ii) the remainder of the plumage is white, instead of varying shades of brown, red or yellow. The second difference is due to the sex-linked dominant *S*

Table 1

	Brown Leghorn	Rhode Island Red	Indian Game	Light Sussex
Comb type	*p p*	*p p*	*P P*	*p p*
Skin colour	*w w*	*w w*	*w w*	*W W*
Plumage colour	*s s*	*s s*	*s s*	*S S*
Plumage pattern (extent of black)	*co co*	*Co Co*	*co co* (modified)	*Co Co*
Ear-lobe colour	white	red	red	red
Body weight (♂♂) g.	2300	3400	3800	3400

p = single; *P* = pea; autosomal
w = yellow; *W* = white; autosomal
s = gold; *S* = silver (white) sex-linked (♂ homogametic)
co = Brown Leghorn pattern; *Co* = Columbian restriction of black; autosomal

Ear-lobe colour shows irregular 'blending' inheritance. The figures for body weight are from the standard weights given by Jull (1940, Table 2); they are intended merely to indicate the order of magnitude of size differences, since there is a great amount of within-breed variation.

(silver), which inhibits most brown, red and yellow pigments, but is without effect on the distribution of black. There are some brownish pigments which are not inhibited by *S* (the salmon breast of the Brown Leghorn female is the most clear-cut example) but there is usually no difficulty in diagnosing *S* either in chick downs or in adult plumage.

Columbian restriction of black has been ascribed (Smyth & Bohren, 1949; Kimball, 1954) to an allele (e^C) of self-black (*E*), recessive to *E*, but dominant to a third allele (e^+) present in Brown Leghorns. Critical evidence for or against allelism is lacking, partly because Columbian does not, or at least does not always, segregate sharply from Brown Leghorn pattern. This is clear, both from past experience (Pease & Cock, 1951) and from the breeding tests reported later in this paper. F_1's usually approximate to Columbian pattern, with black slightly more extensive than in purebred Columbians, but a wide range of types occurs in segregating generations,

with the extent of black varying, often without any clear discontinuity, from typical Columbian, through intermediate grades to Brown Leghorn pattern and sometimes beyond. Similar difficulties arise if chick down instead of adult plumage is taken as the criterion. Columbian breeds have a plain-coloured down (buff or reddish-brown in the absence of *S*, otherwise white) with black pigment confined to the bases of the growing wing-quills, whereas the Brown Leghorn has a broad dark dorsal stripe, with additional lateral stripes on the hips and through the eyes. F_1 downs are plain (although a black spot on the head, or traces of black on the back often occur, usually in females), but in segregating generations all grades appear, from plain downs, through various degrees of 'reduced' and narrow stripes to typical Brown Leghorn downs. The striping may be distinct or more or less blurred, and downs with a mottled instead of a striped pattern occur. There is, moreover, no very close relationship between chick down and adult plumage; the plain downs tend to grow into Columbian, and the broader stripes into Brown Leghorn pattern, but there are exceptions in both directions (cf. Pease and Cock, 1951).

The assumption of a single major autosomal gene (probably imperfect in dominance) for Columbian restriction, with modifying factors blurring the segregation, does seem to be justified. The symbol *Co* is used here without prejudice to the question of allelism with *E*, which is irrelevant to present purposes. There is an admitted element of arbitrariness in classifying progeny for *Co*; classification has been based on adult plumage rather than on chick down.

The above account omits several minor variations in plumage pattern, whose genetic basis is uncertain. These include differences in the intensity and quality of brown-red-yellow colours, e.g. the dark red-brown ground colour of Rhode Island Reds, contrasted with the paler, more yellow colour of F_1 females from Brown Leghorn ♂ × Light Sussex ♀. Again, some female *S*-segregants have the Brown Leghorn distribution of black, but a white instead of a salmon breast; these have been taken to be *co*-types. There is probably a corresponding group among *S*-segregants, with the breast a golden instead of a salmon colour, but the corresponding types in males are presumably indistinguishable, since the breast in *co*-males is black. The pattern of the Indian Game has been ascribed to yet another allele at the *E*-locus, but for present purposes it suffices to regard it as a modified *co*-type.

Cases F1218 and E2785

These are both fully fertile males, with normal male secondary sexual characters and behaviour; normal male genitalia were found at autopsy. They were produced in the course of an experiment on crossing the Brown Leghorn and Light Sussex breeds, carried out by Mr R. W. Hale at the Agricultural Research Institute of Northern Ireland, Hillsborough, County Down. The pen from which they were bred contained females of both breeds, and two males, one of each breed, were placed in the pen on alternate weeks. Progeny of four types (the two pure breeds and their reciprocal F_1's) were thus produced from the same pen. Chicks were pedigreed to individual dams by trap-nesting; in female chicks paternity could be determined from down colour. Sex, when not evident from down colour, was

established by examination of the vent, and all males were normally discarded at hatching time. The dam of both the mosaics is a typical Light Sussex (D229); it is practically certain that they were sired by the Brown Leghorn (D832). Although it is formally possible that the Light Sussex male (D222) was in some way involved in paternity, this seems extremely unlikely, as all deviations in the mosaics from a normal F_1 male phenotype are in a Brown Leghorn direction. However, it was thought advisable to progeny test the Light Sussex male as well as the mosaics and their parents, and Mr Hale very kindly sent these five birds to Edinburgh in August 1958.

F1218

Hatched: 19 March 1958. Killed in emaciated condition 6 February 1959. Post-mortem examination revealed no abnormalities other than a mild nephritis.

Down colour: buff, i.e. like a normal F_1 female. It was in fact taken to be such until the male sex and mosaic skin colour were noticed at 8 weeks of age.

Body weight: 3020 g. (4 September 1958).

Skin colour: left shank rich yellow (*w*); right shank white (*W*). The effect of *W* on the colour of the body skin is slight, but there is a median line of division between white and yellow skin discernible along the keel of the sternum.

Adult plumage: the superficial appearance is closely similar to that of a normal Brown Leghorn male, and there is no perceptible difference between right and left sides. Closer examination reveals a departure from normal Brown Leghorn colouration. This consists in a substitution of white for gold or red-brown in the non-black parts of many of the feathers of the neck and saddle, and of all the wing secondaries. All the feathers so affected have gold colouration at the distal end, which fades gradually into white proximally. There is also a little scattered white in the wing coverts. The white could be regarded either as indicating the presence of silver (*S*) in parts of the plumage, or as merely a somewhat atypical expression of gold (*s*). The symmetrical distribution of white, its confinement to the proximal parts of feathers, and the absence of an abrupt boundary between white and gold within single feathers, all favour the latter possibility. The bulk of the plumage is undoubtedly gold, but the possibility that some small patches of silver are present cannot be ruled out.

Ear-lobes: mainly white, with a slight mottling of red on both sides. (This mottling is characteristic of F_1's, although the relative amounts of white and red vary widely. A little red mottling is sometimes found in pure Brown Leghorns, and is in fact present in D832.)

Skeletal size: the left side is consistently smaller than the right. The average difference in length of the paired bones amounts to 4–5% (Table 2), and the bones of the left side show a corresponding reduction in thickness. Among median bones the asymmetry is less evident. The skull and sternum are both slightly asymmetrical, but none of the vertebrae shows significant asymmetry, and, apart from a slightly shorter ilium on the left, the synsacral complex is remarkably straight and symmetrical.

Table 2. *Bone lengths in cm.*

	F1218			E2785			Cl			Br		
	R	L	$\frac{R-L}{R}$%	L	R	$\frac{L-R}{L}$%	L	R	$\frac{L-R}{L}$%	L	R	$\frac{L-R}{L}$%
Femur	11·1	10·6	4·5	11·4	10·6	6·5	10·1	8·8	13	10·2	9·9	3
Tibiotarus	16·4	15·3	6·5	16·8	15·4	8·5	14·8	14·1	5	14·9	14·6	2
Tarsometatarus	11·2	10·7	5	11·2	10·2	9·5	10·1	9·9	2	10·1	10·0	1·5
Humerus	9·7	9·5	3	9·9	9·2	7·5	9·0	8·9	1	8·9	8·9	1
Radius	9·0	8·7	4	8·9	8·5	5	8·0	—	—	7·8	7·7	0·5
Ulna	9·9	9·5	4	9·9	9·4	5	8·8	—	—	8·8	8·7	1
Carpometacarpus	5·4	5·3	3	5·4	5·2	5	4·7	4·6	1	4·9	4·8	2
Coracoid	7·5	7·0	7	7·6	7·0	7·5	7·0	6·8	2	6·8	6·7	0·5
Scapula	10·0	9·9	1	10·1	9·3	7·5	—	—	—	—	—	—
Clavicles (excl. hypocleideum)	6·0	5·7	4·5	5·9	5·3	10·5	5·8	5·6	2·5	—	—	—
Ilium	12·5	12·1	3	12·1	10·8	10·5	—	—	—	—	—	—

Gaps in the entries under Cl and Br are due to broken bones; these two skeletons were very fragile.
Lengths are given here to the nearest 0·1 cm., but were in fact measured to 0·01 cm. Differences are given to the nearest 0·5%.

E2785

Hatched: 12 March 1958. Died suddenly, 24 April 1959; nephritis was diagnosed.

Down colour: buff, with one white wing.

Body weight: 2900 g. (4 September 1958).

Skin colour: left shank entirely white, right shank pale yellow, with a streak of white running down the back and along one of the toes. In the body skin there is a median line of yellow/white demarcation discernible below the cloaca, but not elsewhere.

Adult plumage: there is a very evident asymmetry in both colour and pattern (Plate I, Figs. 1 and 2). On the ventral side, the division is clear-cut and median, but dorsally it is irregular. The type of the right side extends onto the left of the head, whereas that of the left covers the greater part of both sides of neck and saddle hackles. With this proviso, the two types are as follows. The right side is basically Brown Leghorn type, but, as in F1218, there are minor departures from true Brown Leghorn pattern. The outer edges of the wing-flights are predominantly white, but some have small smudges of gold near the tip, and one primary has a completely gold edge. Analogous whitening may well occur in some of the neck and saddle feathers too; the irregular demarcation between the two types makes certainty about this impossible. Many of the feathers of the ventral plumage and leg (which would be solid black in a normal Brown Leghorn male) have a little brown and/or white mottling near the tip. The plumage of the left side differs in two respects: it is undoubtedly silver (*S*), and it shows a degree of restriction of black intermediate between a typical F_1 and a Brown Leghorn. The surface colour of the breast, ventral abdomen and leg is an irregular mixture of black and white in about equal amounts.

Ear-lobes: predominantly white, with some red mottling on both sides.

Skeletal size: asymmetry is rather more extreme than in F1218, and it is the right side which is smaller (Table 2). The bones of the left side are distinctly thicker as well as longer. The median bones show a more consistent asymmetry than those of F1218. The skull and sternum are both markedly asymmetrical, as are the cervical vertebrae, particularly the more anterior ones. In the thoracic and synsacral regions, the vertebral column has been distorted into a ⌐-shape, so that the 6th and 7th thoracic vertebrae (the 7th being the 1st vertebra of the synsacrum) run practically transversely, with their anterior ends towards the right. This, together with the unequal length of the legs, produced a very clumsy stance and gait. At post-mortem examination, the left lobes of the liver were found to be much larger than the right lobes.

Breeding tests

The following test matings were carried out by artificial insemination at Edinburgh in November 1958–January 1959. Apart from the fact that F1218 became obviously unwell during this period, and then yielded little or no semen, fertility and hatchability were satisfactory.

(1) The Brown Leghorn male D832 was mated to the Light Sussex dam D229. Thirty-eight offspring (17 ♀♀ : 21 ♂♂) survived to the eighteenth day of incubation

Fig. 1.

Fig. 2.

Ventral (Fig. 1) and dorsal (Fig. 2) views of E2785. Ventrally, the line of demarcation between the two plumage types is median and sharp; the apparent deviation towards the right side posteriorly in Fig. 1 is due to exposure of the grey underfluff in the region between the sternum and the cloaca. The dorsal demarcation line is much more irregular and indistinct. There are patches of gold at the sides of the comb, along the right border of the neck hackles, and in the saddle; the difference between gold and black, like that between white and yellow skin, does not reproduce well in black-and-white.

Table 3

(*a*) *Classification for* S *and sex; survivors beyond seventeenth day of incubation*

		S	*s*	Total
F1218	♀♀	9	13	22
	♂♂	15	7	22
	Total	24	20	44
	Dead embryos			10
	Rearing losses			3
	Remaining			31
E2785	♀♀	23	24	47
	♂♂	20	17	37
	Unsexed	—	1	1
	Total	43	42	85
	Dead embryos			11
	Rearing losses			12
	Remaining			62

(*b*) *Classification for* S, W *and sex; survivors beyond 8 weeks*

		S W	*S w*	*s W*	*s w*	Total
F1218	♀♀	3	4	3	4	14
	♂♂	3	9	4	1	17
	Total	6	13	7	5	31
	S : *s*					19 : 12
	W : *w*					13 : 18
E2785	♀♀	7	9	10	8	34
	♂♂	4	11	6	7	28
	Total	11	20	16	15	62
	S : *s*					31 : 31
	W : *w*					27 : 35

(*c*) *Classification for extent of black in plumage; survivors beyond 8 weeks*

	Near F_1 pattern (*Co*)	Intermediate (*Co*?)	Near Brown Leghorn pattern (*co*)	Total
F1218	7	9	15	31
E2785	12	15	35	62

Offspring of F1218 and E2785 mated to Brown Leghorn hens (*s*, *w*, *co*). Most of the rearing losses are due to a power failure affecting the brooders. Segregation of extent of black in plumage shows no significant association with *S*, *W* or sex.

or beyond; all males were silver (*S*) and all females gold (*s*). Twenty-seven of these (10♀♀ : 17 ♂♂) were reared to 8 weeks or beyond (when reliable diagnosis of skin-colour is first possible); all were white-skinned (*W*). In addition, eight F_1 and two pure Light Sussex females from D229 had been reared at Hillsborough; these again were all normal.

The plumage pattern of the left side of E2785 differs from a normal F_1 in that black pigment is too extensive. As the degree of restriction of black may show some variation in different F_1 families, the normal sibs afford a critical comparison on this point. In all of them (both sexes), black was only slightly more extensive than in pure Light Sussex; there were always some black markings in the saddle, and usually traces of scattered black in the ventral plumage, but the latter were always small in extent. The normal range of variation in this mating is narrow, and the right side of E2785 lies well beyond it.

(2) The Light Sussex male D222 was mated to Brown Leghorn hens from the Poultry Research Centre flock. Seventy-eight offspring (37♀♀ : 41 ♂♂) were obtained, all silver (*S*). Of these, forty-seven were reared to 8 weeks or beyond, all being white-skinned (*W*). The extent of black in the plumage was similar to that in the offspring of mating (1), and showed only slight variations. This male is thus homozygous for the breed characters of the Light Sussex, which makes it even more difficult to imagine that he was in any way involved in the paternity of the mosaics.

(3) The two mosaics were each mated to Brown Leghorn hens; the results are summarized in Table 3. The most striking aspect is the clear evidence that F1218 carries *S* and *Co*, despite his being phenotypically *s* and *co* on both sides. There is, in fact, nothing in the results from either mosaic to distinguish them from normal F_1's. None of the segregation ratios for sex, *W* or *S* departs significantly from 1 : 1. There is no indication of gonadal mosaicism; the *ws* class, which should then be in excess, is actually below expectation in both cases. If, as is probable, the intermediate class in respect of restriction of black is to be regarded as modified *Co*, the segregation ratios for *Co* also approximate to 1 : 1.

Case Cl

This, like the following case (Br), was studied while the writer was on the staff of the Poultry Genetics Unit, School of Agriculture, University of Cambridge, and was sent in by a private breeder. It was received in August 1954, at the age of about 6 months. Information as to parentage is limited to the fact that it derived from a cross of Indian Game and Light Sussex. The breeder stated that it was probably not an F_1, but rather an F_2 or backcross. Death occurred in June 1956, and autopsy disclosed extensive tumours in the liver, kidneys and mesenteries. Normal male genitalia were found, and the secondary sexual characters were at all times those of a normal male.

Comb type: pea (*P*).

Skin colour: right shank white (*W*), left shank yellow. No difference in the colour of the body skin of the two sides could be detected. The intense reddening of the mid-ventral skin of this bird would obscure a possible line of yellow/white demar-

cation. (Such reddening is common among Indian Game, due to the short and rather sparse plumage, which leaves the mid-ventral skin exposed.)

Adult plumage: silver Columbian type, but black pigment more extensive than in pure Light Sussex. The two sides differ in this respect, black being slightly but definitely more extensive on the right. The difference is noticeable in the wing-flights and in the breast, which is practically pure white on the left, but has irregular black spangling on the right.

Skeletal size: there is a general reduction in size on the left side, but different bones are not equally affected (Table 2). Whereas the bones of the wing show only a slight and barely significant reduction in length of about 1%, the femur is reduced by 13%, although the reduction becomes progressively less in the more distal leg bones. This is not accompanied by any corresponding reduction in thickness; the shafts of the right femur and tibiotarsus are respectively 15% and 8% thicker (absolutely) than those of the left. In contrast to F1218 and E2785, the two sides of the body thus differ markedly in proportions as well as in size. The ratio between the lengths of tarsometatarsus and femur is 1·12 on the right and 0·99 on the left; comparison with Hutt's (1929) mean value of 0·992 (S.D. 0·014) indicates that it is the smaller right side whose proportions are abnormal. The sternum is concave towards the right, and the skull is slightly asymmetrical, but the vertebral column and synsacrum are practically symmetrical.

Breeding test: mated in 1955 with Brown Leghorn hens, he yielded 126 offspring, all silver (*S*). Good 1 : 1 ratios were obtained for comb-type (63 *P* : 63 *p*), sex (56 ♀♀ : 55 ♂♂, dead embryos not sexed) and skin colour (52 *W* : 50 *w*). Approximately one-half of the offspring had Columbian or modified Columbian plumage pattern; the segregation was fairly sharp in males (29 *Co* : 20 *co*) but very indistinct in females. These results accord with a genotype of *S*/*S*; *P*/*p*; *W*/*w*; *Co*/*co*, and give no suggestion of gonadal mosaicism.

Attempts were made in 1956 to obtain offspring from the two testes separately, by everting the cloaca during massage and sucking semen into a pipette as it emerged from the genital papilla. Some semen was obtained in this way, but this, like all other semen collected in 1956, proved to be infertile.

In an attempt to detect a possible red-cell mosaicism, the blood of Cl was tested with three lytic sera kindly supplied by Professor P. B. Medawar. These tests yield no evidence of mosaicism, but failure to detect it with these particular sera does not prove it to be absent.

Case Br

This is an F_1 male (Rhode Island Red ♂ × Light Sussex ♀) received in April 1955 at about 14 weeks of age. It appeared from the start a weakly and underdeveloped individual, and died in August of the same year. Autopsy showed extensive ulceration of the inner surface of the crop. The genitalia were very small for a bird of this age (testes 14 mm. long, 6 mm. diameter) but otherwise apparently normal and entirely male. This was rather surprising, as the secondary sexual characters had been predominantly female. Comb and wattles had remained small and there were no signs of spur growth. Inactive testes could easily account for this, but the

plumage structure was predominantly female, although there were some feathers of male or intermediate type among the wing-coverts. Female-type plumage normally develops only in the presence of ovarian hormone or excess thyroid hormone, but since there was no sign of hermaphroditism in the genitalia, it seems that this was probably in some way due to the persistent poor health, and unconnected with the mosaicism. It is not impossible that the bird carried the henny-feathering gene; there is no evidence to refute or support this.

Skin colour: white (*W*) on the right, yellow on the left, with a clear mid-ventral line of demarcation between white and yellow.

Adult plumage: the plumage pattern is that normal for a male of this cross (i.e. Silver Columbian, with scattered splashes of chestnut) and there is no perceptible asymmetry.

Skeletal size: there is a rather slight reduction in size on the left side (Table 2). There is a tendency, as in case Cl, for the femur and tibiotarsus to be more strongly affected than other limb bones, but this is much less marked. Sternum, thoracic vertebrae and synsacrum all show a curvature, concave on the left.

DISCUSSION

There is no compelling reason to regard either Cl or Br as the result of anything more complicated than loss of the *W*-bearing autosome from one nucleus at first cleavage division; the slight difference in plumage pattern in Cl could easily be due to a modifying gene linked with *W*. However, this explanation rests merely on the absence of contradictory evidence, and can be no more than tentative. The abnormal proportions of the smaller side of Cl recall analogous changes in three earlier cases (Cock, 1955) which were also mosaic for *W*. Changes in size and proportions would be a not unexpected consequence of aneuploidy, but it is difficult to see why they should be extreme in some cases, but very slight in others involving the same locus. According to Newcomer (1957) the fowl has only five pairs of autosomes, corresponding with the five known autosomal linkage groups, plus an indefinite number of small 'chromosomoids'. Despite extensive tests (Warren, 1949) no linkage of *W* with any other gene has yet been detected, which suggests that it may be carried by one of the chromosomoids.

Before considering the possible mode of origin of F1218 and E2785 it is necessary to deal with several points which seem to depend on irregularities in the distribution of descendants of the two original cells. Previous cases have shown departures from a strict half-and-half distribution in the melanocytes (see especially Greenwood & Blyth, 1951, and Jerome & Huntsman, 1959) and in the germ-cells (Greenwood & Blyth, 1951; Blyth, 1954); in view of the migratory embryonic history of melanoblasts and of primitive germ-cells this is not surprising. In E2785 the distribution of the melanocytes, although broadly half-and-half, clearly shows some degree of irregularity. In F1218 neither *S* nor *Co* is expressed in the plumage, but both are present in the gonads; the assumption of an extreme degree of irregularity, so that all or most of the melanocytes of both sides are derived from the left half of the early

embryo, seems to be the only way of reconciling these facts. The breeding results of both cases (and of Cl) are most plausibly interpreted as reflecting irregularities in the origin of the germ-cells, the recessive side of the body being represented, if at all, in numbers too small to cause a significant disturbance of segregation ratios. There is also evidence of departures from a strict half-and-half distribution in the skin (the white patch on the right shank of E2785) and in skeletal tissues (the relatively symmetrical vertebrae and synsacrum of F1218).

With these considerations in mind, F1218 and E2785 are more similar than first appearances suggest. Each has one half (the right in F1218, the left in E2785) whose constitution at least approaches that of the normal F_1, whereas, the other half resembles the paternal Brown Leghorn, both in the reduced size and in the lack of maternal dominants, *S*, *W* and *Co*. Only two types of explanation of this condition seem even remotely possible. The first is that one side of the body is of purely paternal origin, the other side being derived by a normal fertilization process. The paternal side would presumably be diploid; this could result either from a mitosis with the final stages suppressed, or from the fusion of the nuclei of two supernumerary spermatozoa. The second possible explanation is that a normal fertilization has been followed by multiple maldistribution of the chromosomes at first cleavage division; maldistribution is intended to cover such possibilities as elimination of part or whole chromosomes, non-disjunction, translocation, somatic crossing over.

The hypothesis of purely paternal origin is capable of explaining several previous half-and-half fowl mosaics (Cock, 1955), notably the two cases of Greenwood & Blyth (1951), which show important similarities with the present cases. In addition, several pigeons with irregular (i.e. no sign of a half-and-half arrangement) and restricted pigmentation mosaicism (Hollander, 1949) and one irregular mosaic fowl (Cock, 1959) can be explained in a similar way. In the present cases, although the main features are readily accounted for, there are certain obstacles to the acceptance of this hypothesis.

In both cases the smaller side shows deviations of a relatively minor nature from strict Brown Leghorn phenotype in adult plumage pattern; these might perhaps (although not very plausibly) be explained away, e.g. as the result of islands of silver melanocytes. A more serious difficulty is the fact that the down pattern in both cases was the plain buff characteristic of the F_1 female, without the striping of the pure Brown Leghorn. In the case of F1218 this observation was made before the mosaic nature of the bird was recognized, but Mr Hale assures me that the down certainly was of the unstriped F_1 type, and points out that a down of Brown Leghorn type from this dam would immediately have aroused suspicions of a pedigreeing error. (That an unstriped down should be followed by adult plumage close to Brown Leghorn type is not in itself exceptional; several such cases occurred among the progeny—see Introduction.) A further difficulty is that in E2785 the plumage of the left side has much more black than a normal F_1. Taken singly, any one of these difficulties might be neglected or explained away, but in conjunction they constitute a serious obstacle to the hypothesis of purely paternal origin.

The second type of explanation, that of multiple maldistribution of the chromosomes, demands a remarkable degree of coincidence, particularly in view of the similarities of the two cases. Since *W* and *Co* are not known to be linked, it would seem that at least two autosomes are involved in addition to the X-chromosome, and in E2785 the departure of the right side from normal F_1 phenotype suggests loss from this side of a third autosome carrying a modifier of *Co*. There are serious difficulties in such an explanation, quite apart from questions of coincidence.

(1) Somatic crossing-over is excluded (at least as the sole cause of the mosaicism) by the breeding results; it would require the larger side to be homozygous for *S* and *W*, so that *S w* and *s W* types should not appear among the progeny. All other forms of maldistribution would lead to aneuploidy on one or both sides, and it is difficult to believe that multiply aneuploid tissue would be viable, even if, as is possible, both *W* and *Co* are carried on the small 'chromosomoids' of Newcomer (1957).

(2) The chromosomal imbalance involved in aneuploidy, even when not so severe as to be lethal, should lead to disturbances in growth, and abnormalities of proportions on the smaller side have in fact been prominent features of several previous cases (including Cl) mosaic for *W*. There is no sign of similar abnormalities in F1218 or E2785; the variation in degree of reduction in length of different limb bones is relatively slight and does not follow a similar pattern in the two birds.

(3) The normal males gonads are not incompatible with the assumption that one side of the body is XX and the other X-, since the breeding results suggest that all or most of the germ-cells are derived from the XX side. However, in several previous cases which appear on other evidence to have been XX/X- mosaics there has been an average difference of 13 to 15% (i.e. the normal ♂ versus ♀ difference) in the length of the limb bones of the two sides (Cock, 1955, Table 6). The corresponding differences in the present cases are considerably less than this (5% in F1218; 7% in E2785), whereas maldistribution of the autosomes should have increased rather than reduced the difference.

The mode of origin of F1218 and E2785 must therefore remain enigmatic; neither of the two types of explanation considered above is convincing, and yet there seems to be no conceivable third alternative. The aura of uncertainty might have been reduced by cytological comparison of somatic tissues from the two sides; this had been intended but was prevented by the premature death of both birds. The occurrence of two cases in the same family is probably not purely coincidental but reflects an underlying predisposition to mosaicism. In the present instance this might be a non-inherited peculiarity of one or other parent, but mosaics which were descended from a mosaic parent or grandparent, indicating a hereditary tendency, have been reported by Crew & Munro (1939) and by Blyth (1954).

SUMMARY

Two mosaic birds are described, from a sex-linked F_1 cross (Rhode Island Red ♂ × Light Sussex ♀). They are sexually normal males and full brothers. Each has the following abnormalities on one side of the body: absence of the maternal dominants *S* (silver, sex-linked), *W* (white skin) and *Co* (Columbian restriction of black), and

reduction in size. In one case all the melanocytes appear to be derived from the smaller side, so that the plumage pattern is identical on the two sides; in the other there is a minor deviation (modified expression of *Co*) from normal F_1 plumage pattern on the larger side. Mated to Brown Leghorn hens, both mosaics bred as normal F_1 males, with no evidence of germinal mosaicism. Over thirty full sibs were all found to be normal.

A purely paternal origin of the smaller side appears to be excluded in both cases by the lack of striping in the down, and by minor deviations from normal Brown Leghorn phenotype in adult plumage pattern. Origin by some kind of maldistribution of chromosomes at first cleavage division would require the X-chromosome and possibly as many as three autosomes to be affected simultaneously.

Two further unrelated crossbred mosaic males are described. Each has yellow skin (*w*) on one side of the body. In one case this is accompanied by a slight difference in plumage pattern, reduction in size and abnormal proportions in the limb bones; in the other merely by a slight reduction in size. Loss or non-disjunction of a single chromosome at first cleavage division is a possible explanation of these.

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The ecological genetics of growth in *Drosophila*

1. BODY SIZE AND DEVELOPMENTAL TIME ON DIFFERENT DIETS

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1. INTRODUCTION

The interrelations between environment and the phenotypic expression of genetic differences have not received the attention they merit. Laboratory studies in the quantitative inheritance of *Drosophila* have so far shed little light on the problem, due either to choice of character or experimental conditions. Such favourite characters as the numbers of sternital or sternopleural bristles are comparatively unaffected by gross environmental variation and are unsuitable material for studying gene-environment interaction. On the other hand, growth, measured in terms of body size and duration of the developmental period, is very sensitive to environmental variation and offers therefore ideal material. But in earlier studies on the inheritance of body size in Drosophila (Robertson & Reeve, 1952; Robertson, 1955, 1957), nutrition and temperature were kept as uniformly favourable as possible to simplify the analysis. Nevertheless, the evidence and concepts from this earlier work provide a basis for the next stage in which genetic behaviour is studied in different controlled environments.

As soon as we select for apparently the same 'character' in different conditions we meet the problem of how far qualitatively different physiological changes contribute to the response. For example, Falconer & Latyszewski (1952) showed that mice, selected for body size on high and low planes of nutrition, differ in carcase composition. Waddington (1957) has demonstrated how sub-threshold effects may be brought to light by appropriate stimuli and then selected for. Also (Waddington, 1959), when *Drosophila* larvae were grown for some twenty generations on media with high salt concentration, the size of the anal papillae—believed to function in osmotic regulation—was increased above the normal level even on the ordinary medium while the responsiveness to increasing salt concentration was also enhanced. I have shown, from a comparative survey of the cellular make-up of the *Drosophila* wing (Robertson, 1959*b*, *c*), that the way in which growth is apportioned between cell size and cell number differs according to the nature of the environmental variation and also the kind of genetic change which is imposed on a population. Qualitatively different causes may lead to outwardly similar variation in wing or

body size, although closer inspection often reveals characteristic contrasts in development. Clearly, therefore, changes in physiology and development must be regarded as essential information in a general study of gene-environment interaction.

Such information is likely to prove relevant to the rate of change and also how far the response to selection can be pushed in a given direction. It is evident from the various selection experiments reported over the last decade or so, that the statistical parameters derived from the correlations between relatives are of low predictive value in selected populations and the origin of the so-called 'plateau', which marks the end of selection response, remains obscure. A comparative study of the level at which selection response falls off in different controlled conditions, coupled with parallel records of physiological changes, could set these problems in a fresh light and suggest new ways of interpreting familiar data.

Thus, the statistical techniques and concepts which have been developed for the analysis of quantitative variation must be combined with physiological description and analysis in a more comprehensive approach. Statistical variation between individuals must ultimately be interpreted in biological terms, and this can hardly be avoided, without generating inscrutable confusion, when different environments are involved. At present, quantitative and physiological genetics are kept in separate compartments to their mutual disadvantage. I suggest that a deliberate breaking-down of barriers is long overdue to clear the way for a greater variety of experimental analysis which will lead to new data and fresh concepts.

In planning a systematic attack along these lines, the ecology of the animal provides an essential guide to the kind of environmental variation which first merits attention, since there are so many ways in which conditions can be altered. Any species or population is normally subject to an array of environmental conditions, which have a certain average consistency to which the population is adapted. By reference to ecology we can create, in the laboratory, environmental conditions which are normally encountered by many or few individuals in a population or which are perhaps only experienced from time to time by the population as a whole. A comparison of the level of phenotypic variation, selection response and its physiological attributes is likely to be instructive under suitable contrasted conditions. Also, by following in the laboratory genetic and physiological changes which accompany adaptation to different kinds of environment, we may be able to detect regularities which can be related to the differences between geographical races or species living under different conditions. Thus, the analysis can be progressively broadened to embrace in a wider synthesis data from field ecology, including breeding structure and effective population size.

Since the procedure advocated here does not fall neatly within the confines of what are currently termed quantitative, physiological or population genetics, but exploits the techniques and concepts of all of them to a common end, it is useful to have a descriptive label. Professor Waddington has suggested to me that 'ecological genetics' might do. This has the merit of emphasizing the animal's relation to its environment as the point of departure and I have therefore adopted it.

The present introductory paper is the first of a series in which this approach is devoted to the genetics of growth in *Drosophila melanogaster*. It deals generally with methods of comparing and interpreting differences in growth on different diets.

2. MATERIALS AND METHODS

For this—or any other species—environmental variation is often largely a question of variation in the quantity and chemical composition of the diet during the stages of growth and reproduction. Genetic differences is response to nutritional conditions might be anticipated, but the relative importance and properties of such variation, both within and between populations, is unknown. Hence comparisons of growth on different controlled diets provide the obvious starting point.

With *Drosophila* the study of gene-environment interaction entails the use of chemically defined, aseptic media in place of the heterogeneous complex of yeast and other micro-organisms on which the animal is normally cultured. Various workers have contributed to the perfection of such chemically defined media, especially Tatum (1939), Schultz, St Lawrence & Newmeyer (1946), Begg & Robertson (1950), Hinton, Noyes & Ellis (1951) and Sang (1956), who has determined the quantitative requirements of essential nutrients. Since shortage of any essential constituent, or an unfavourable balance, especially of amino-acids, will lengthen the larval period and may reduce final body size as well, there are many ways of preparing an unfavourable diet. Of chief interest is the sort of nutritional variation commonly encountered in nature. This is unknown, but circumstantial evidence suggests that the choice of suitable conditions for comparing genotypes need not be entirely a matter of guesswork. Thus Sang (1959) concluded, from various published estimates of the composition of yeast upon which *Drosophila* mainly feeds, that B vitamins, with the possible exception of folic acid, are unlikely to be limiting factors under natural conditions. Hence media deficient in protein and/or yeast nucleic acid, probably offer the most realistic choice of sub-optimal conditions to begin with. As information accumulates, it should be ultimately possible to state more precisely the principal attributes of environmental variation in nature, population cage or culture bottle.

The media upon which larvae were grown aseptically comprise either Medium C of Sang (1956) or various modifications of it. The complete medium contains vitamin-free casein, fructose, yeast nucleic acid (RNA), cholesterol, lecithin, salts and seven B vitamins in an agar gel. The modified diets involve (*a*) omission of the fructose, (*b*) reduction of the RNA level from 0·36% to 0·09%, (*c*) reduction of the casein concentration from 5% to 2%.

After the medium had been autoclaved, eggs, which had been dechorionated and freed of micro-organisms by repeated washing in various antiseptic fluids and sterile water, were introduced, forty at a time, into each culture tube. Four or five replicates per genotype and treatment were set up in this way. Body size has been recorded generally on eight females per culture, by measuring thorax length on the live flies in the manner described elsewhere (Robertson & Reeve, 1952). For live yeast cultures the usual maize-meal molasses medium has been used, fortified with

dried yeast and seeded with live baker's yeast. All experiments have been carried out at 25°C.

Development time is based on the morning and evening count of the adults hatching in individual cultures. The duration of the pupal period is comparatively independent of genetic differences in body size as well as nutritional variation during the larval period and is about 4·3 days at 25°C., as Sang (1956) has noted. Hence this value has been subtracted from the total period of development to estimate the larval period more precisely. In comparisons between strains reared on live media, the duration of the larval stage is, of course, appreciably shorter than on sub-optimal media, and so, in most cases, newly emerged larvae have been set up in about fifteen small tubes—10 larvae per tube—to provide greater accuracy and more replicates without undertaking the labour of recording pupation time on individual larvae. The average development time in the egg is added to the estimates of larval life to make them comparable with the other data.

The basic records have been transformed to a log scale. Body size is expressed as three times the natural logarithm of thorax length, in hundredths of a millimetre. Unpublished tests have shown that a unit change on this scale corresponds closely with a unit change in the log weight of newly emerged flies. As we shall see, there is good internal evidence that the log scale is the most appropriate for biological interpretation of the data, while there is the additional advantage that differences between means on the log scale can be converted roughly into percentage differences by multiplying by 100, and this helps the reader to keep the relative magnitude of such differences in perspective. Development time, or, more exactly, duration of larval life, is expressed as log days. Since development time was recorded for all the flies hatching from the cultures, whereas body size was recorded on a random sample of them, the degrees of freedom for mean development time is almost always considerably higher than for body size; only the data for females have been analysed.

The wild population used in these tests is known as *Pacific*; it was derived from a large number of wild flies and had been run for about a year in a population cage before the tests began. To provide material for comparison, a large and small strain have been created by selection on the ordinary live yeast medium. The procedure was essentially the same as that used in earlier long-term experiments (Robertson, 1955). The thorax length of twenty pairs of flies from each of five replicate cultures was recorded and the extreme four pairs combined to give a total of twenty pairs selected from 100 pairs examined in each generation. The experiment was stopped after seven generations. Flies of the unselected, wild population were also reared each generation.

3. RESULTS

(*a*) *The effects of selection*

Figure 1 shows the effects of selection, expressed as deviation from the mean of the unselected flies. The points refer to the tests carried out after 2, 3, 4 and 6 rounds of selection on live yeast medium and the flies to which the points refer were the grandchildren of selected flies. Comparing their deviations with the F_1 of

selected flies showed that a generation of mass mating did not lead to appreciable change and so the points shown in the figure accurately represent the course of selection.

Selection, as usual, quickly separated out two distinct populations which, by generation 6, differed by some 30% in body size. Judged by the deviations from

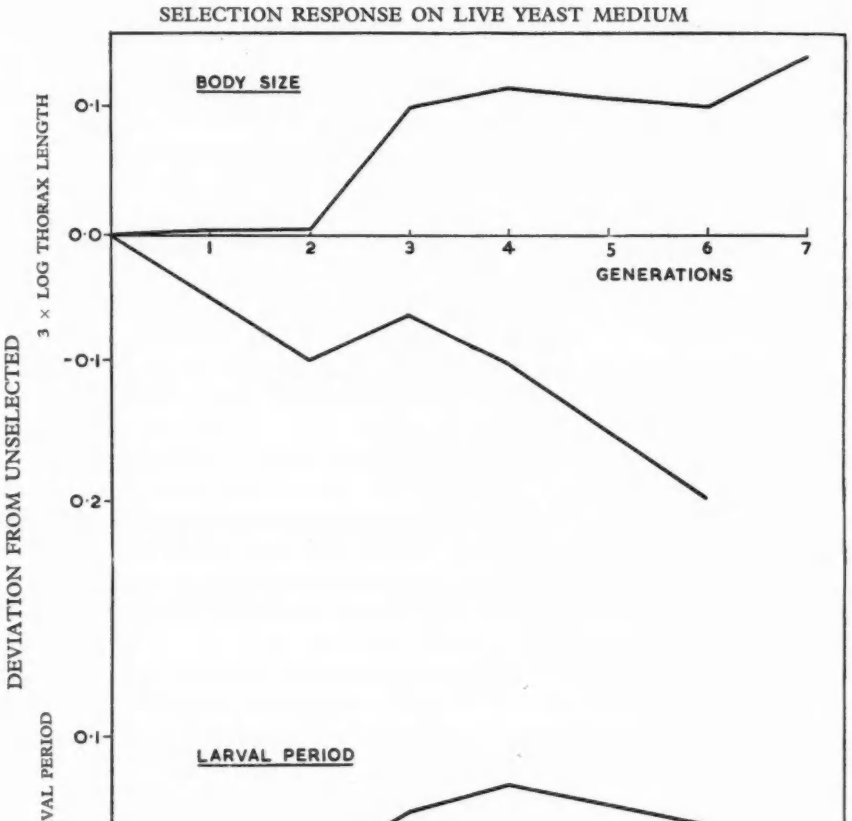

Fig. 1.

unselected, selection for large body size was apparently ineffective until generation 3; an alternative explanation will be given later. The strains also came to differ in development time—the larger strain taking longer to develop. This difference is

chiefly due to change on the part of the large strain, and the small strain does not differ consistently from the unselected flies. This difference in duration of larval period is quickly established and shows little evidence of progressive increase as body size diverges; that is indicated in Table 1.

Table 1. *Divergence between selected strains in body size and development time; log units*

Generation	Body size	Larval period
2	0·11	0·04
3	0·17	0·01
4	0·22	0·05
6	0·32	0·06

It is quite clear that the positive relation between body size and development time which may be inferred from the variation between wild flies or the first generation or so of selection would be quite misleading as a guide to later changes due to selection; this point will be taken up again later.

(*b*) *Reaction of the unselected strain to different diets*

Before considering the effects of rearing different strains on alternative media, it is helpful to get an idea of the order of effect of these media on growth and for this purpose the behaviour of the unselected flies can be taken as a guide. These were grown on the live yeast medium and on the three aseptic media—without fructose, with low RNA, and with low protein concentration—as part of the tests carried out after 2, 3, 4 and 6 generations of selection. There is no reason to anticipate that genetic differences will contribute to differences between tests and so comparison of performance at different times will provide a measure of ability to reproduce a particular environment as well as indicating the order of effect of the different treatments. Table 2 sets out the variance analysis for these four tests; the following points must be noted.

(i) The media lacking or deficient in fructose, RNA and protein cause approximately 10%, 20% and 30% reduction in body size, and some 30%, 50% and 70% lengthening in the duration of larval life.

(ii) Variance between replicated cultures within tests is quite unimportant for body size, except on the low-protein medium. For larval period, the culture effects are just over the 0·05 level of significance for the fructose- and RNA-deficient media, but are clearly significant for low protein.

(iii) Significant heterogeneity between tests was generally present, and since genetic differences are unlikely, this calls for comment. Heterogeneity between tests on the ordinary live yeast medium is not surprising. It is worth noting in the case of body size, that the heterogeneity, although significant, is not very great, whereas it is very marked in the duration of larval period. This is a good illustration of the tendency for sub-optimal conditions, provided they are not too severe, to be accompanied by a lengthening of development with little or no reduction of body size, i.e.

there is a well-marked capacity for regulating body size by prolonging the larval period (Sang, 1956; Robertson, 1959*a*). Further evidence on this phenomenon will be presented in the next paper. Such heterogeneity in the larval period also shows how variable the live yeast medium may be, even though we try to make it uniformly favourable.

On the aseptic media, such differences in repeat tests are at first sight unexpected. Temperature was held constant, and variation due to temperature differences can be ruled out anyway on internal evidence based on the lack of correlation between body size on contrasted media in successive tests. Other observations suggest that such differences in performance are probably due to variation in autoclaving tem-

Table 2. *Comparison of repeated tests with the unselected population on alternative media*

	Mean of all tests—log units			
	Live yeast	No fructose	Low RNA	Low protein
Body size	14·08	13·98	13·85	13·75
Larval period	1·43	1·79	1·94	2·12

	Variance analysis—mean squares × 10^2							
	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square
				Body size				
Between tests	3	2·39*	3	3·39*	3	1·34	3	16·75**
Between cultures	40	0·46	13	0·47	13	0·24	13	2·26**
Within cultures	132	0·39	110	0·52	111	0·55	108	0·77
				Larval period				
Between tests	3	148·0**	3	0·1	3	18·7**	3	389·0**
Between cultures	64	0·5	14	1·4*	13	1·0*	13	2·1**
Within cultures	253	0·6	250	0·6	189	0·4	243	0·6

* ** indicate significance at the 0·05 and 0·01 level of probability.

perature, since sub-optimal media are generally rendered less adequate by prolonging the time of autoclaving, and such an effect will be relatively more marked the more sub-optimal the diet; hence the greatest evidence of heterogeneity in the low-protein medium. Evans & Butts (1949) have shown that certain amino-acids are inactivated when autoclaved in the presence of sugar and it is possible that some such reaction is involved here. With the equipment available it is practically impossible to ensure that the total heat exposure of the medium will be identical in successive tests, although the autoclaving time is carefully standardized. Also, heterogeneity between cultures could arise from uneven heating due to temperature gradients within the autoclave. However, such heterogeneity between tests is really quite minor compared with the differences between treatments and, as we shall shortly discover, can in fact be turned to advantage to bring to light an unsuspected phenomenon, which would have been missed if such heterogeneity had not occurred.

(iv) With respect to the within-culture variance of body size, representing the combined effects of genetic segregation and irreducible environmental variation, the lowest value is found on the live yeast medium (0·0039), it rises on the fructose deficient and low-RNA media to respectively 0·0052 and 0·0055, and to the still higher value of 0·0077 on the least favourable, protein-deficient diet. According to Bartlett's test (1937), such differences in variance are highly significant ($\chi^2 = 330$ for 3 degrees of freedom, $p < 0{\cdot}001$). In view of the chemical and physical homogeneity of the synthetic medium, there is little doubt that the greater within-culture variance represents an increase in the phenotypic expression of gene differences. This could derive from a kind of scalar transformation such that a unit difference under optimal conditions is represented by more than a unit difference as the mean falls or, on the other hand—and much more likely—from the segregation of genetic differences which make little or no contribution to the variance under favourable conditions but do so when the diet is sub-optimal, to the extent of doubling the variance on the low-protein diet. In development time there is virtually no difference between treatments in the within-culture variance. This may partly reflect the much lower precision with which development time is recorded, compared with body size, rather than a real difference in the behaviour of these two measures of growth.

(*c*) *Reaction of selected strains to different diets*

We can now turn to the problem of whether or not the genetically different strains behave in the same way when exposed to different conditions, using body size as the criterion of performance. Since the data are expressed in logarithms, absence of gene-environment interaction will be reflected in the same difference in mean—within sampling limits—when the strains are compared on alternative media. Table 3 sets out the mean deviations for body size on live yeast and on the alternative deficient media for unselected flies, for the large and small strains and also for the cross between them. With regard to the latter, reciprocal matings were made and eggs set up separately, but since maternal effects were apparently absent or very slight, the data from the progeny of reciprocal crosses have been combined. The *F*-values quoted below the sets of differences refer to tests of heterogenity of the differences between means for flies grown on the live and on the appropriate sub-optimal diet. The error variance for each set is twice the variance of a mean based on the pooled variance for live yeast and whatever other treatment is involved. This procedure is open to some objection due to the heterogeneity of the within-culture variances, but this is not so great as to make the tests seriously misleading. Generally where interaction exists, it is obvious from mere inspection of the data.

In the comparisons at generation 2, 3 and 4, only on the low-RNA medium at generation 2 is there unequivocal evidence of interaction, although the *F*-values are consistently high for the differences between the live yeast and the fructose-deficient medium, and almost certainly indicate genuine differences in reaction. But by generation 6 there are really striking contrasts in response to the alternative diets. Thus strain differences in gene-environment interaction are clearly established after only a few generations of mass selection.

There is no very obvious regularity about the table of differences with respect to treatment and direction of selection, except perhaps for the 10% greater reduction of the large strain on low protein at generation 6, compared with controls or small

Table 3. *Differences between strains in average body size (log units) of flies reared on live yeast medium and alternative sub-optimal diets*

Reduction in body size below that on live yeast medium

Genotype	No fructose	Low RNA	Low casein
	Generation 2		
Unselected	0·08	0·23	0·29
Large	0·03	0·16	0·26
Small	0·04	0·20	0·26
Cross	0·05	0·15	0·27
F	1·8	6·8**	<1
	Generation 3		
Unselected	0·11	0·21	0·27
Large	0·07	0·22	0·26
Small	0·09	0·25	0·23
Cross	0·12	0·25	0·21
F	1·9	1·0	1·2
	Generation 4		
Unselected	0·12	0·19	0·44
Large	0·12	0·20	0·44
Small	0·11	0·19	0·45
Cross	0·05	0·19	0·47
F	1·8	<1	<1
	Generation 6		
Unselected	0·07	0·28	0·24
Large	0·07	0·23	0·34
Small	0·00	0·28	0·24
Cross	0·08	0·28	0·30
F	4·7**	1·5	5·5**

** indicates significance at the 0·01 level of probability.

strain. This recalls the similar reaction of other large strains after five generations of selection (Robertson, 1959*a*).

However, the properties of these gene-environment differences cannot be understood merely from records of body size; development time must also be taken into account. In searching for a possible relationship between body size and duration of the larval period, the data from the live yeast cultures are of little use, since they fall on the horizontal part of the curve where great variation in larval period leaves body size comparatively unchanged. The low-protein diet is apparently much more affected by autoclaving than the other sub-optimal media and this may involve an additional complication. Hence the media which either lack fructose or have a low

RNA content have been used for comparing body size with development time; it will be recalled that these media result in some 10% and 20% reduction of body size along with about 40% and 50% increase in larval period.

Since there is heterogeneity between tests, the differences in body size and larval period between flies grown on the fructose-deficient and the low-RNA media in successive tests vary. What is the relationship between the two measures of growth

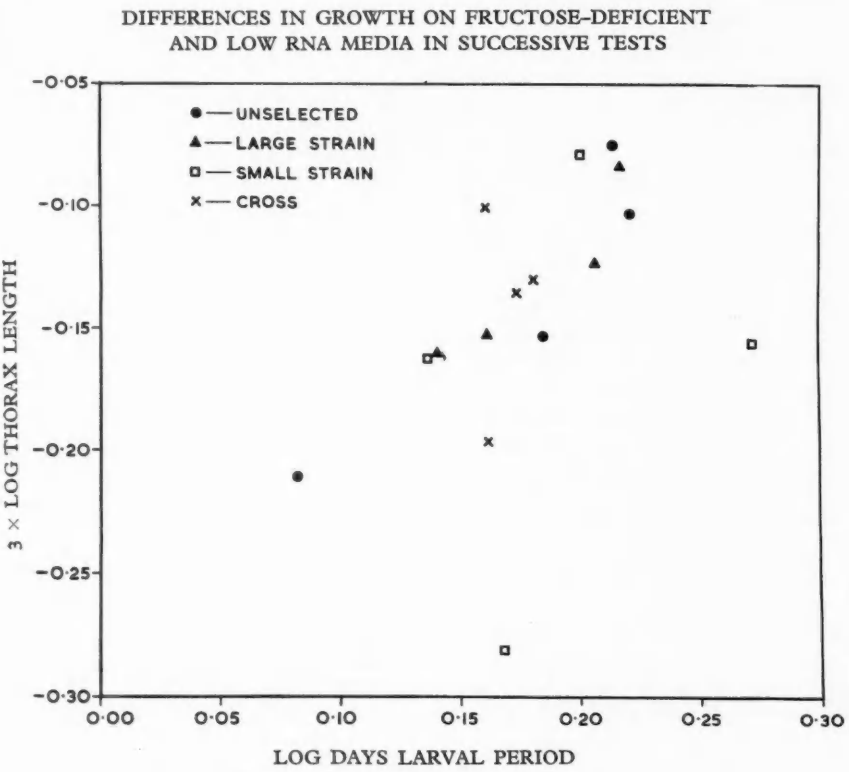

Fig. 2. Body size and larval period are expressed as deviations from performance on the fructose-deficient medium.

for such variation in the difference between the two media? The answer turns out to be both unequivocal and a little unexpected. In Fig. 2 the differences in body size, expressed as a deviation from the larger flies grown on fructose-deficient medium, are plotted against the corresponding differences for development time for the four tests. There is evidently a strong correlation; the smaller the differences in larval period, the greater the difference in body size between flies grown on the two media. Only two points stand apart from the rest; these refer to the small strain at generations 3 and 6. Excluding all four values relating to the small strain, to avoid bias, the regression of differences in body size upon differences in larval period works out

at 0·75 ± 0·26—a value not far off unity. Thus the observed value is just the opposite of what might be expected from the comparison of differences between means, averaged for all tests, in Table 2. Along with the reaction to gross nutritional differences, there is this evidence of an inverse relationship with respect to minor changes. The range of differences in body size, compatible with this positive regression, is about 0·15 log units, which is equivalent to about twice the within-culture standard deviation on these media.

The basis for this relationship is clarified in Fig. 3, which shows mean body size and development time of the unselected flies grown on the pair of alternative media

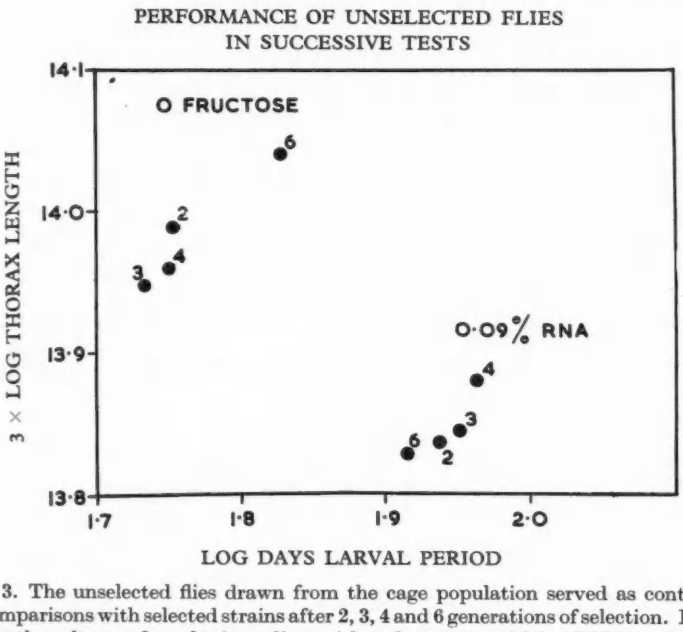

Fig. 3. The unselected flies drawn from the cage population served as controls in comparisons with selected strains after 2, 3, 4 and 6 generations of selection. In each test the cultures of synthetic medium with no fructose or with low RNA content were all autoclaved together.

in the four tests. For the differences between successive tests on the same medium, the regression of body size on development time is 0·92 ± 0·19, and 0·98 ± 0·37 for the fructose-deficient and low-RNA diets respectively. Hence the minor differences in final composition of each type of medium, previously attributed to uncontrolled variation in heat exposure during autoclaving, are associated with a positive regression of body size on larval period, such that the ratio of body size to duration of larval period remains constant. This relationship, characteristic of minor differences in the composition of the diet, is in sharp contrast with the negative regression of body size on development time when major differences exist; the average regression in the latter case is close to −1 on the log scale.

It is worth noting that the ranking of mean body size in tests at different generations is 6, 2, 4 and 3 on the fructose-deficient medium, compared with 4, 3, 2 and 6

for the low-RNA series; i.e. the order is more or less reversed. This may mean that temperature-labile effects on nutritional imbalance, which favour relatively larger size and longer development time on the fructose-deficient medium, have the reverse effect when the level of RNA is reduced.

The kind of direct comparisons carried out on the unselected flies in the four tests cannot be applied to the selected strains, since their average size is steadily changing with selection; hence the need to deal in differences within tests, as in Fig. 2. Since all the latter comparisons, except two tests with the small strain, fall into line with the controls, this apparent plasticity of response to minor nutritional variation seems well established. Its distinguishing feature is the constancy of the ratio of body size to development time. When the variation in diet becomes too great or alters in some specific way, this ratio declines since the lengthening of development time is accompanied by smaller, not larger, body size. Thus the relations between the two aspects of growth can be used to distinguish and study different kinds of gene-environment interaction. If only body size were recorded, the existence of general gene-environment interaction could be recognized, but could not be analysed further. It is likely that the expression of this kind of response, which involves a constant 'growth rate', varies genetically. Hence individuals may react to an identical difference in nutrition by taking relatively longer or shorter time to develop and by growing to correspondingly larger or smaller size. Behaviour of this sort probably contributes to the gene-environment interaction which has been detected in comparisons between strains grown on different media. Superimposed on such variation in response are the effects of differences in the level of adaptation to sub-optimal diets which will be reflected in a sharp decline in 'growth rate'. When we compare different strains on different types of diet these two kinds of reaction are intermixed and it will require further experiments to evaluate their relative importance.

Such considerations immediately raise the question whether responses which lead to a positive correlation between body size and larval period commonly occur in live yeast cultures, and in particular, whether this is relevant to the genetic correlation of these two measures which exists among individuals of wild populations (Reeve, 1954; Robertson, 1957), and for which we have evidence in the present selection experiment. This seems likely from the following argument. It was shown in Fig. 1 that two generations of selection for large size were apparently without effect when selection response was measured as deviation from unselected on the live yeast medium. But is it quite clear that selection had in fact led to a significant shift of genotype, since body size is significantly and consistently greater on the three sub-optimal media. This is shown in Table 4.

It also looks as if the deviation of the small strain, on live yeast medium, is over-estimated as much as the large strain deviation is underestimated. If the unselected flies reacted to prevailing conditions in the live yeast cultures by growing relatively bigger and taking longer to develop than either of the selected strains, the observed discrepancy can be accounted for.

Hence the initial selection for large and small size favours genotypes which respond

to nutritional conditions in opposite ways, and therefore fluctuation in conditions from generation to generation will lead to variation in the estimates of how much progress selection has effected. How far body size can be increased or diminished by picking out genotypes which are responsible for characteristically different or opposite reactions to the diet will depend in part at least on how far nutritional

Table 4. *Deviation in body size from unselected after two generations of selection; log units*

Strains	Media			
	Live yeast	No fructose	Low RNA	Low protein
Large	0·01	0·05	0·08	0·04
Small	−0·10	−0·06	−0·07	−0·07
Total difference between large and small strains	0·11	0·11	0·15	0·11

conditions can be reproduced in successive generations. In the present test, selection for this kind of change appears to have been comparatively ineffective and the major changes of body size due to selection are more or less independent of the duration of the larval period.

DISCUSSION

When the diet is sub-optimal the larval period is prolonged but final body size is not necessarily reduced below the maximum at a given temperature. There is therefore a well-marked ability to regulate body size in the presence of such adverse conditions, but when the diet becomes too deficient for one or more essential nutrients, body size is reduced as well. Also, when larvae are grown on increasingly poorer diets there is a distinct tendency for a given percentage reduction in final body size to be associated with an equivalent proportional lengthening of the larval period. It is to be expected therefore that genetic differences in reaction to the larval diet will be revealed not only in the degree of reduction in body size or lengthening of larval period but also in the variable capacity to regulate body size when the larval diet is sub-optimal. Evidence on this point will be presented in later papers of this series.

This basic pattern of response suggests a systematic approach to genetic differences in reaction to similar conditions. Earlier work (Robertson, 1959*a*) has shown that a few generations of selection for either large or small body size generally results in greater proportional reduction of adult body size on deficient diets than is true for unselected individuals. A similar situation developed after six generations of selection for large body size in the present selection experiment. How far such genetic differences in response vary according to the particular kind of nutritional deficiency or imbalance is an interesting topic for future study. It is quite clear, however, that genetic differences in reaction to nutritional variation are widespread in the population. Changes in body size due to selection are no doubt especially likely to involve different growth responses in relation to diet, although it remains to be seen whether selection for other characters reveals any regularity in this respect.

There do not appear to be any precise data about how much of the observed variation of body size in wild flies in nature can be attributed to nutritional variation, after allowing for temperature fluctuation. Sokoloff (1957) has suggested that food shortage is unimportant because the body size of wild flies does not vary greatly. However, as we have seen, the record of body size alone may be a poor guide to the adequacy or otherwise of the larval food supply, since regulation may lead to longer development time without much effect on body size. Also it might be expected that species will differ considerably in this capacity for maintaining a constant body size. But whatever the situation in nature, there is no doubt about the importance of nutritional variation in the laboratory cultures of *Drosophila* either in bottle or population cage.

The reaction of the growing larva to its diet is not confined solely to maintaining a genetically predetermined size or suffering a characteristic decline in the size at which pupation occurs. It appears that the duration of the growing period may also vary so that a longer growing period leads to larger body size and the converse. The clearest evidence for this reaction is provided by the successive tests on the media deficient in fructose and RNA. Judging from the behaviour of the wild stock, differences between repeat tests were such that larger body size was regularly associated with proportionately longer larval period. This was quite unexpected, since crude variation of the diet, as between say media deficient in fructose and RNA, leads to a negative correlation between these measures of growth—the longer the development time, the smaller the fly.

The essential causes of the differences in repeat tests are unknown, but possible origins may be suggested in diminishing order of likelihood. They may arise from uncontrollable differences in the heat exposure of the synthetic media during autoclaving, so that although the successive batches of a given medium start the same, they end up slightly different, due to heat-labile reactions. The tendency for the order of ranking of body size in the four tests to be reversed on the different media suggests that variation in heat exposure leads to inverse changes in the concentration or availability of one or more critical nutrients, according to the initial composition of the medium. Alternatively, the differences may be due to a maternal effect, whereby the diet of the mother can influence the growth of the progeny. Or conceivably, differences in the timing of critical stages of growth in relation to diurnal rhythm may play a part.

But, whatever the final explanation, there is evidently a plasticity of response in relation to environmental variation which is expressed by parallel changes in body size and development time. Such a plasticity of response may in turn reflect a buffering system which favours a characteristic ratio between final size and duration of the growth period. If it can be shown that genotypes, which are atypical with respect to the normal gene arrays in a population, are relatively less successful in maintaining such a characteristic ratio, this reaction could provide an empirical measure of physiological homeostasis. This problem is being examined further.

This type of growth response is probably relevant to the positive correlation between body size and development time among wild flies grown on the live yeast

medium. Genetic differences which determine whether individuals react to prevailing conditions by growing for relatively longer or shorter periods to become correspondingly bigger or smaller may be expected to contribute to selection response. Also the extent to which, say, larger body size can be attained by this kind of change depends on the repeatability of the appropriate conditions in successive generations. If these vary, consistent selection for this kind of change will be comparatively ineffective and the selection response will depend on changes which alter growth rate but not duration of the growth period.

Gene-environment interaction was invoked to account for the discrepancy between the estimates of selection response on the live yeast medium, on one hand, and various synthetic media, on the other. It was suggested that the unselected individuals had reacted via relatively longer development time and larger body size on the live yeast medium, compared with either of the selected strains, whereas on the synthetic media the three populations were more alike in this respect. Both from these comparisons and from the repeat tests on the media deficient in fructose and RNA, it appears that the range of body size associated with this kind of reaction is equivalent at least to between one and two times the within-culture standard deviation.

A general comment is relevant here. Had the data in these experiments been confined to the 'character' body size alone, this interesting phenomenon would not have been detected, since it would have been impossible to distinguish differences in growth response under different conditions. The heterogeneity between repeat tests would have been classified as unavoidable error variance, whereas, by taking development time into account, such variation is deprived of some of its apparently irreducible confusion and fresh possibilities of analysis and interpretation appear. In particular, the extent to which body size may be increased either by altering the growth rate or by increasing the duration of the growing period, the genetic behaviour and reaction to environment of strains differentiated in this way, together with the physiological interrelations between such alternative kinds of change, raise problems of great interest which indicate some of the next steps in this study.

Finally, when wild flies are grown on media deficient in essential nutrients, the within-culture variance between individuals increases. For example, on the low-protein diet it was approximately twice as great as on live yeast medium. The greater variance under adverse conditions may be attributed to the segregation of genetic differences which are indistinguishable when the diet is improved. This raises the question of how far parallel selection for, say, large body size on such different diets, leads to qualitatively different physiological changes and characteristically different reactions to controlled changes in the environment. Experiments bearing on this problem will be described in succeeding papers.

SUMMARY

1. The interrelations between environment and the phenotypic expression of genetic differences have not received the attention they merit. Laboratory studies in quantitative inheritance, either by choice of character or experimental conditions,

have not shed much light on this problem. Selection for the same character in different environments is likely to involve qualitative differences in physiology and development. Comparative study of such changes will throw light on the genetics of development generally, which in turn is relevant to how far the selection response can be pushed in a given direction. Since statistical variation between individuals must ultimately be interpreted in biological terms, the unnatural barriers between quantitative and physiological genetics must be broken down to clear the way for a greater variety of experimental analysis and a more widely based approach to the interpretation of individual differences in populations. The ecology of the animal provides the point of departure and guide to the kind of environmental variation which should be studied first. Since the suggested approach cuts across the conventional limits of quantitative, physiological and population genetics and exploits the concepts and methods of these alternative approaches to a common end, it is convenient to have a descriptive label. The term 'ecological genetics' has been adopted.

2. This introductory paper is the first of a series dealing with experiments orientated along these lines. Since environmental variation largely consists of variation in the quantity and composition of the diet, the growth of individuals from a cage population of *Drosophila melanogaster* and also other strains has been studied on a variety of aseptic, synthetic diets. Body size and duration of the larval period are taken as measures of growth. There is a well-marked ability to regulate body size, by extending the duration of development, provided the diet is not too deficient. When the diet is further reduced development time is further lengthened and body size is reduced as well.

3. To test for genetic differences in reaction to the diet, strains have been created by selecting for large or small body size, and their performance, together with that of the cross between them, has been compared with the performance of unselected individuals on alternative diets for the first few generations of mass selection. There is evidence of gene-environment interaction quite early in selection, and after six generations striking differences were detected. It is concluded that genetic differences in reaction to different sub-optimal diets are widespread in the population.

4. The within-culture variance is increased by growing larvae on progressively more deficient diets and is approximately twice as great on a low-protein diet as on the usual live yeast medium. This increase is attributed to the segregation of genetic differences which are unimportant and contribute little to the variance under more favourable conditions.

5. Comparison of body size and development time in repeated tests with two diets lacking fructose or deficient in ribonucleic acid revealed evidence of a plasticity of response to minor nutritional variation which is characterized by a positive association between body size and the duration of the growth period. This relationship is the reverse of that associated with crude variation in the diet which leads to a negative association between development time and body size. This plasticity of response probably represents an aspect of physiological homeostasis. Genetic differences in the magnitude and direction of this response probably contribute to

gene-environment interaction generally, and this probably accounts for apparent discrepancies in alternative estimates of the response to selection for large and small body size when these are based on deviations from the unselected. This suggests the need for determining how far body size may be increased either by altering the growth rate or by extending the growth period, and also how far strains differentiated in such respects differ in their reaction to controlled differences in nutrition.

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The ecological genetics of growth in *Drosophila*

2. SELECTION FOR LARGE BODY SIZE ON DIFFERENT DIETS

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1. INTRODUCTION

When larvae from a wild population of *Drosophila* are grown on diets deficient in one or more essential nutrients, the within-culture variance of the body size of the adult flies is greater than on optimal diets. This increase has been attributed, in part at least, to the segregation of genetic differences which make little or no contribution under more favourable conditions (see the preceding paper). Hence the physiological changes which underlie the selection response for the same 'character', body size, are likely to vary according to the nature of the environment, although mere record of body size in a single set of conditions can throw no light on this possibility, which is relevant to the rate and extent of the response to selection and also the properties of the genetic variation of body size generally. The present paper describes the results of selecting for larger body size in environments which differ in the quality of the diet supplied to the larvae.

2. MATERIALS AND METHODS

A cage population of *Drosophila melanogaster*, known as *Pacific*, has been used in these tests. The preceding, introductory paper (Robertson, 1960) described the culture of larvae on aseptic, synthetic media and also the reasons for choosing a log scale for both body size and the duration of larval life. Body size is expressed as three times the natural logarithm of thorax length, measured in $\frac{1}{100}$ mm. A unit difference on this scale corresponds to a unit difference in log weight.

The synthetic media used in two of the selection experiments were: (i) a protein-deficient medium with 2% casein in place of 5% in the 'complete' Medium C of Sang (1956), and (ii) Medium C diluted to one-third normal strength of all constituents, except agar. It so happens that the two media reduce body size by about the same amount—some 25%—and while both considerably increase the larval period, the low-protein diet lengthens it more than the diluted medium does. In addition, two strains have been selected on the usual maize-meal medium, fortified with dried and seeded with live yeast. These were started at the same time and run in parallel. Evidence is also drawn from a short-term selection experiment on the live yeast medium, which was described in the preceding paper.

The selection procedure differed in one respect according to diet. For the two parallel strains on the live yeast medium, three pairs of flies were selected from each

of ten pairs from five replicate cultures, to make fifteen pairs chosen from a total of fifty pairs examined. On the aseptic media, an average of thirty pairs of flies were chosen each generation from about 130 pairs examined. This difference in population size—twice as great on the sterile media—was dictated by the need to collect sufficient eggs from the parent flies for an adequate series of aseptic cultures. Since these flies were reduced in size, their egg production was correspondingly lower. Unselected flies from the foundation population were regularly cultured for comparison with the flies grown on the live yeast medium. In addition unselected larvae were grown on the alternative aseptic media, while larvae from the strains selected on the sub-optimal diets were also grown on the ordinary medium, sufficiently often to see how the deviation from the performance of the unselected stock varied with diet.

3. RESULTS

(*a*) *Selection on the live yeast medium*

Figure 1 shows the response to selection in the pair of lines selected for large body size. They represent the same general features as quoted for the effects of mass selection in other populations (Robertson, 1955). The graph suggests that selection

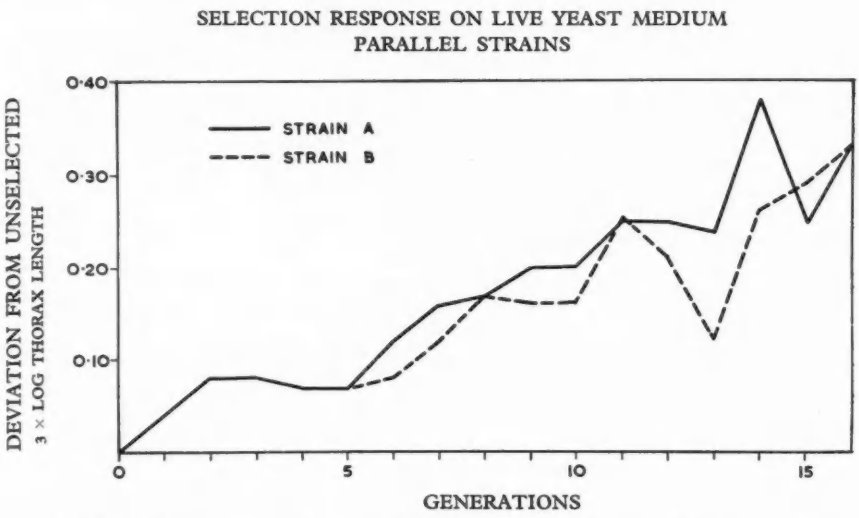

Fig. 1. The two strains were selected for large body size on the ordinary live yeast medium. The average deviations from unselected were almost identical for the strains for the first five generations of selection.

was still effective after sixteen generations of selection when the experiments were discontinued. By this time the strains were some 30% bigger than the unselected flies. The general similarity of the selection response in the two lines, especially during the first five generations, is really quite remarkable.

(b) Selection on the low-protein diet

Figure 2 shows the selection response on the low-protein diet as deviations from unselected. Comparisons on both the aseptic and the live yeast medium are shown. On the low-protein diet, body size continued to change until generation 7 when progress ceased abruptly, and, apart from an unexplained decline at generation 9—almost certainly an artefact—the curve failed to rise appreciably during a further

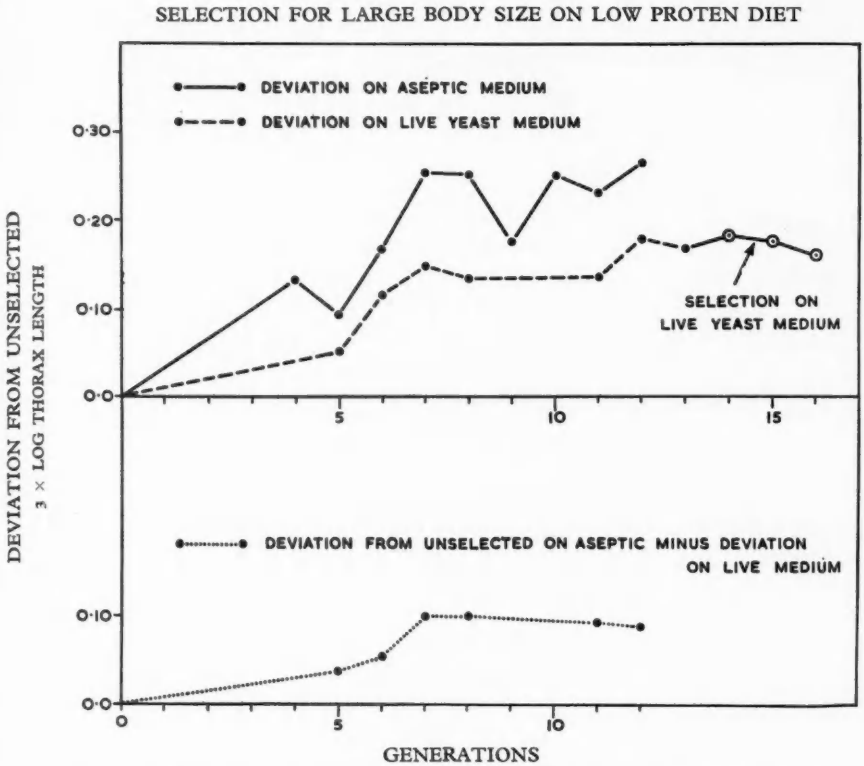

Fig. 2. The effects of selection on the synthetic, low-protein diet. After twelve generations selection was continued on the live yeast medium.

five generations, after which selection on the low-protein diet was discontinued. At generations 5 through 8 and also 11 and 12, progeny of the selected flies were also grown on the live yeast medium and their deviations from unselected are also shown.

Two features stand out. Firstly the deviation from unselected, on the log scale, is consistently less on the live yeast than on the low-protein diet. It appears that selection has picked out genotypes which are better adapted to the sub-optimal diet but which do not contribute to larger size on the favourable live yeast medium. The contribution of such effects to the total selection response can be estimated by subtracting the deviations from controls for the live yeast medium from the

corresponding deviations for the low-protein diet and such differences are shown in the lower graph of Fig. 2. Selection has improved performance on the low protein diet by some 10%. The rest of the response appears to be due to changes which are expressed on both low-protein and the live yeast media.

This result can be compared with the effects of growing large strains selected on live yeast medium on the same low-protein diet (Robertson, 1959, 1960). After a

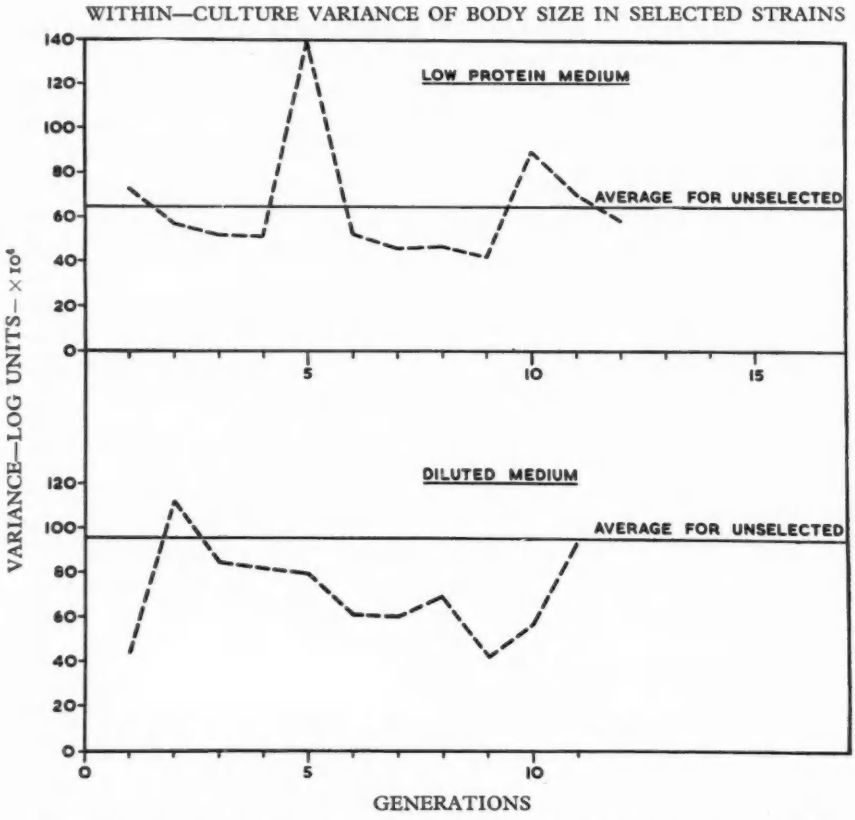

Fig. 3. The average within-culture variance for the unselected flies represents the pooled variance for the controls set up during the selection experiments together with data from other tests in which the same medium was used.

few generations, performance on the low-protein diet declines so that the deviation from unselected becomes less. Thus parallel selection for the same 'character'—body size—on different larval diets leads to different reactions to similar nutritional changes. It would be quite easy to produce large strains which deviate equally from unselected on the live yeast medium but which behave quite differently when the diet is altered.

The second notable feature of these results is the earliness with which the plateau

is reached—progress stops after only seven generations. In other mass selection experiments for large body size on the ordinary medium, the response extended over a longer period and attained a greater proportional increase over the foundation population (Robertson, 1955). Also, in the pair of lines selected from the Pacific population, selection response was much greater (Fig. 1). It is worth noting that the loss of variance due to restricted population size in successive generations should be greater, other things being equal, in the strains which responded most to selection, since there were only fifteen pairs of parents per generation, compared with thirty pairs on the aseptic medium.

The within-culture variance of body size tends to decline with effective selection on the low-protein diet. Figure 3 shows the variance in successive generations, compared with the average value for the wild population reared on the same medium. For the latter estimate, all available data have been combined, including data from tests not dealt with here. The variance declines over the first four generations or so and then remains fairly steady, apart from a jump at generation 6, which is probably due to infection of the synthetic cultures. This decline in variance is attributed to better adaptation to the sub-optimal diet which is accompanied by a reduction in the effects of segregation and recombination. The tendency for the variance to rise about the time the plateau is reached may be significant, and will be noted later.

Direct evidence for improved adaptation to the low-protein diet is provided by records of the duration of the larval period, which has been recorded at intervals; these are set out in Table 1.

Table 1. *Duration of the larval period of the strain selected on low protein*

	Deviation from unselected—log days	
Generation	Low-protein medium	Live yeast medium
4	−0·06*	0·01
6	−0·04*	0·01
8	−0·11*	0·05
9	0·01	—
12	−0·01	0·04*

Asterisks indicate significance at the 0·05 level of probability.

During the early period of selection, development time is significantly shortened in the strain selected on low protein. From the evidence given in the preceding paper, genetic changes which lead to larger body size and shorter duration of the larval period can only mean that growth is that much less affected by limiting factors in the diet. There is no evidence that such changes also shorten development time on the favourable medium, as might be expected, although there is some tendency for development time to be slightly increased at the end of the selection period, and this could imply some lowering of adaptation to the live yeast diet. On the low-protein medium, differences between selected and unselected in the larval period are later obliterated, due possibly to increasing importance of other kinds of change —characteristically expressed on the live yeast medium as well—which become

increasingly incompatible with the increase of body size via improved adaptation to the diet. If this is so, with suitable modifications of selection procedure, it might be possible to increase performance, and hence body size, on the low-protein diet to a higher level than was possible here; such changes might be expected either to leave average body size on the live yeast medium unchanged or to lower it. This point will be discussed again later in relation to the origin of the plateau on different media.

It could be argued that culture on the low-protein synthetic diet is a particularly effective way of revealing genetic differences so that selection would be relatively more discriminating and lead thereby to a greater rate of fixation which might contribute to an earlier fall-off in response, compared with the live yeast medium. It is true that there is a decline in variance, but there is every reason to attribute this primarily to qualitative change in the gene arrays rather than to increasing homozygosity. There is additional circumstantial evidence that different rates of fixation can hardly be invoked to account for these characteristic differences in selection response. It was noted that the level of egg production is strikingly higher in the selected than in the unselected strain. At generation 4, adult performance of selected and unselected flies reared on the low-protein medium was recorded over the 4 days of peak production, while at this and also the fifth and seventh generations, the performance of flies grown on the live yeast medium was recorded. In the test at generation 4, for larvae grown on low protein, the selected flies laid some 30% more eggs than the unselected, over the test period—a highly significant difference. Part of this difference could be attributed to the improved adaptation to the sub-optimal diet, discussed above, which had become effectively less sub-optimal for them than for the controls. This would naturally result in higher egg production, since variation in body size due to larval nutrition is highly correlated with the rate of egg production (Robertson, 1957). But a difference of this magnitude suggests that intrinsic egg production was also higher, and this was confirmed in the three comparisons between selected and unselected flies grown on the ordinary medium. Here the selected flies laid some 15% more eggs—again, statistically, a highly significant difference. It is well known that egg production is sensitive to loss of heterozygosity, and if selection on the synthetic media had contributed appreciably to the level of inbreeding, we should hardly expect egg production to increase. How far this striking correlated response is peculiar to the conditions in which selection was carried out, is not yet clear and provides material for further study. Finally it is worth noting that records of viability from egg to adult showed little evidence of change and certainly no decline in the selected strains.

Hence the early plateau on the low-protein diet is essentially due to incompatibility between alternative effects which promote larger body size by different kinds of change in growth and metabolism. At first, such different effects may behave more or less independently, in contributing to selection response, but a few generations of selection create a situation in which potential increase in body size in one way involves changes which would reduce it in other ways and so the response stops, since body size is the sole criterion of selection. This interpretation does not conflict with the earlier evidence that selection, for the kinds of effect which are expressed

on the live yeast medium, is associated with greater proportional reduction on low protein diets.

It could be argued that if the strains selected on low protein were transferred to the favourable, live yeast medium, the physiological incompatibility would no longer exist, since the genetic changes which improve performance on the low-protein diet would be irrelevant under these conditions. The potential reserves of genetic variation, which lead to larger body size on the live yeast medium, would become accessible and allow selection to continue. To test this, after generation 12 selection was carried out on the live medium for a further four generations. No increase in size occurred beyond the level characteristic for this medium during the plateau period. It may be inferred that the genetic changes effected by selection on low protein are not neutral with respect to growth on the live yeast medium. Either they inhibit the expression of genetic differences which otherwise would contribute to the variance, or, and perhaps more likely, selection on the low-protein diet has lowered adaptation to the live yeast medium and established the conditions for mutual incompatibility between alternative pathways which promote larger body size. To realize the potential reserves of variation it would be necessary to recreate the right conditions. This would involve undoing the work of selection on the low-protein diet, which would entail a reduction of body size before selection could be re-established with some prospect of reaching the levels found in strains selected from the beginning on the ordinary medium.

(c) *Selection on the diluted medium*

Selection procedure on this medium was identical with that for the low-protein diet. Figure 4 shows the steady response until generation 8, when progress ceased

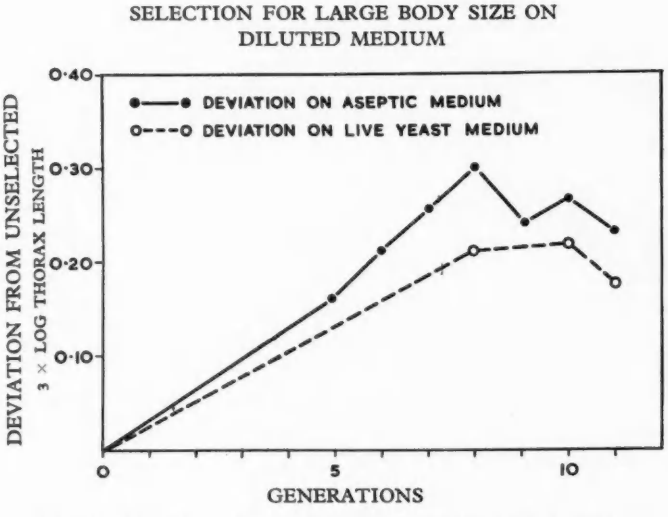

Fig. 4. The effects of selection on the synthetic, diluted medium.

and the strain thereafter fluctuated about a level equivalent to 0·25 log units above unselected. Thus the selection response here is very similar to that on low protein, both with respect to the level of the plateau and the number of generations required to reach it. There is a difference, however, in that the deviation from unselected on the live yeast medium is relatively greater than in the other line. The within-culture variance also declines with selection and shows a tendency to rise after the plateau has been established. Thus the two separate tests on qualitatively different sub-optimal diets present essentially the same pattern of response.

(*d*) *Heritability estimates*

Since we have now the response to selection for large body size in three different environments, we can compare the estimates of heritability. As the deviations from controls were not scored for the first few generations on the diluted medium, the data from generations 5, 6 and 7 for all lines are used for the estimates. Response to selection is taken as the average deviation from unselected for these three generations on the medium used in the selection. Heritability is estimated from the ratio of response to cumulated selection differential (Woolf; see Falconer, 1955); the estimates have been averaged for the three generations in question. The cumulated selection differential is simply the sum of the average deviations of selected flies from their culture means for the preceding generations of selection, i.e. it refers to the total selection pressure applied up to the generation in question. For the live yeast medium, the estimate quoted in Table 2 refers to the average of the values from three large strains—the two selected in parallel and also the large strain used in the experiments described in the first paper of this series. Table 2 shows the various comparisons.

Table 2. *Heritability on different media*

Medium	Deviation from unselected	Cumulated selection differential	Heritability
Live yeast	0·102	0·343	0·30
Low protein	0·176	0·504	0·35
Diluted	0·209	0·548	0·38

The selection response, in terms of deviation from unselected, is greatest on the diluted medium and least on the live yeast. But the cumulated selection differential is least in the latter and considerably greater on both the sub-optimal media, reflecting the greater within-culture variance which has been noted earlier. The heritability estimates are quite similar for the three comparisons. Whether or not this means that sub-optimal conditions also inflate the developmental variance remains to be seen and this possibility is being studied in further tests. It is equally possible that the relative contributions of additive versus non-additive effects also vary according to diet. The fact remains that the similarity between the estimates of heritability does not also apply to the subsequent performance of the strains. On the live yeast medium the predictive value is high, while on the other media it is nil,

since the response to selection ceased immediately after the generations to which these estimates apply.

(*e*) *Genetic correlation*

Falconer (1952) has discussed the problem of how to handle the relations between the response to selection for the same 'character', say body size, in two different environments, such as high and low planes of nutrition, and the expression of the differences due to selection when each strain is reared in the environment other than that used for its selection. Thus for each treatment we have a measure of the direct response and a correlated response from the performance of the strains selected in the alternative conditions. This so-called correlated response can be treated formally as a case of genetic correlation which can be estimated from the equation:

$$r_G = \frac{\text{correlated response}}{\text{direct response}} \cdot \frac{h_1}{h_2} \cdot \frac{\bar{i}_1}{\bar{i}_2},$$

where h_1 and h_2 refer to the square roots of the heritabilities on the alternative conditions; h_1^2 is the heritability in the environment in which the direct response is measured. $\bar{i}_1$ and $\bar{i}_2$ represent the corresponding estimates of selection intensity, namely the average selection differential divided by the average within-culture standard deviation. For each set of comparisons there are two estimates of genetic correlation which should agree if the assumptions which underlie this statistical procedure are valid.

The present experiments provide sufficient data for such estimates. The heritabilities are the same as those quoted in Table 2. The strain reported on in the preceding paper has been used to provide records of performance on sub-optimal diets of a large strain selected on live yeast. Tests on alternative media were carried out during selection and, for the low-protein medium, the data from tests after 4, 6 and 7 generations of selection have been averaged. For performance of this strain on the diluted medium we have only one comparison, at generation 7. For the strain selected on low protein, performance on the live yeast medium is represented by the average of the values recorded for generations 5 to 7. For the strain selected on diluted medium, corresponding values have been estimated from Fig. 4.

Table 3 shows the values used in the estimation of the genetic correlations. There is a considerable lack of consistency between the alternative estimates. Since the heritabilities and the average selection differentials are rather similar for the different strains, the discrepancies are due to the characteristic differences in the growth of the different strains on the alternative media. In all cases the deviation from unselected is less on the alternative medium which is used to estimate the correlated response. This difference is specially striking when the strain selected on live yeast is grown on the diluted medium.

It is worth noting that these discrepancies arose after only a few generations of mass selection. There is evidently little prospect of reliable extrapolation from past to future performance since the computed values of the 'genetic correlation' will vary rather violently in successive generations of selection. Thus, in the first few

generations of selection on the live yeast medium, culture on low protein makes little difference to the proportional deviation from unselected. This was noted in the preceding paper (Robertson, 1960). But as selection proceeds, a substantial deviation may be converted into a low, zero or possibly, even a negative deviation on sub-optimal diets. Such apparent contradictions in the estimates of genetic correlation are to be expected if selection for the same 'character' under dissimilar environmental conditions involves qualitatively different changes in growth which

Table 3. *Correlated response on different media, deviations from unselected, log units*

	Media		
	Live yeast	Low protein	Diluted
Direct response	0·10	0·17	0·21
Correlated response			
Live yeast	—	0·08	0·02
Low protein	0·11	—	—
Diluted	0·16	—	—
h	0·57	0·59	0·62
$\bar{i}$	7·61	6·70	6·40
r_G	1·15		1·70
	0·45		0·09

lead to characteristic differences in reaction when the environment is altered in a particular way. The practical utility of the statistical procedure is open to question if it has such low predictive value and confounds relevant biological differences in a single numerical score.

4. DISCUSSION

These experiments support the view that different nutritional conditions during growth bring different gene arrays into prominence. Although adult body size may be the single criterion of selection, individual variation in different conditions represents, to greater or lesser degree, the effects of genetic segregation and recombination on different processes of growth and metabolism. Hence when environmental conditions are sufficiently different, it is misleading to refer to the 'character' body size as if it were a kind of ultimate category. By suitable control of the environment, selection can be used systematically as a tool to alter growth in different ways. By including records of development time, the reaction to controlled differences in diet and any other criteria which turn out to be relevant, genetic variation in body size, which at first appears amenable only to statistical description and analysis, can be studied in new ways in which genetic behaviour and physiological effect are considered together.

On the sub-optimal diets used here, part of the response to selection for larger body size represents improved adaptation to these conditions. Direct support for this view is provided by the decline of the within-culture variance during the early stages of selection, the shorter development time and also the higher egg production

of the low-protein strain when compared with unselected flies grown on the same medium. On the other hand, there is no evidence in these nor in any other experiments to suggest that selection for larger body size on the live yeast medium represents better adaptation to such conditions. Such differences in the kinds of change which accompany ostensibly parallel selection in different environments are obviously related to differences in how far selection can change the phenotype.

If apparently similar increases of body size can depend on different modifications of growth, it is unlikely that the possibility of substantial change in one direction will be independent of changes in other possible directions. In particular, it was suggested that continued improvement in adaptation to the sub-optimal diets soon becomes incompatible with further change of the kind normally selected for on the live yeast medium, but which also contributes to the variation of body size on the sub-optimal diets. This is consistent with the later rise in the within-culture variance after the period of steady decline, since selection may finally result in some loss of adaptation to the sub-optimal diet and so lead to greater evidence of genetic segregation. The so-called plateau originates when alternative ways of increasing body size, which are initially more or less independent in action, become negatively correlated in effect. The nature of the diet largely determines how far body size can be increased before the impasse is reached. A more extensive comparative study of selection response on different diets would be instructive, since it should throw light on the conditions and selection procedure, for a given population size, best suited for sustained response. It would be rather surprising if the results of uncritical selection for large body size on the live yeast medium could not be improved upon with a better understanding of how growth may be altered in different ways to produce apparently similar variation in final size.

When the strain which had ceased to respond on the low protein was transferred to the live yeast medium, body size did not increase with further selection and remained well below the level reached by strains selected on the live yeast medium. It can hardly be accepted that seven generations of mass selection, with double the population size, will have exhausted the genetic variation which normally contributes to the response under favourable conditions. The apparent contradiction probably rests on the fact that selection on the sub-optimal diet has created a new genetic situation in which it is impossible to gauge the amount of further progress from the performance of strains selected on the live yeast medium from the beginning. Possibly selection on the sub-optimal diet has lowered performance on the live medium and potential variation which favours larger size could only be exploited by undoing the work of selection on the other media.

It is known from other experiments (Robertson, 1959, 1960) that selection for larger body size on the live yeast medium entails, sooner or later, relatively greater reduction in body size—compared with unselected flies—when larvae are grown on protein-deficient or diluted media. It might be argued that a genetically controlled tendency to attain larger body size would automatically raise the protein requirements above the level required for smaller strains. However, such a simple explanation will not do. *A priori* there is no reason why the larval period should not be

extended under such adverse conditions, so that the proportional decline in body size remains the same. Also, strains selected for smaller body size may decline relatively more on sub-optimal diets as well. So it rather looks as if adaptation to the specific composition of the diet, such as amino-acid balance for example, may be relatively more important than crude differences in protein content. This problem will require further study since it suggests ways of defining the essential features and more important limiting factors in the nutritional conditions to which the species is adapted. These considerations must be borne in mind when the response to selection for the same character is being compared in populations adapted to different conditions, and this will be especially true of comparisons between species. Observed differences in the pattern of response may be thought to depend on purely genetic causes such as linkage relations or chromosome number when in fact they stem from unequal differences between the conditions in which selection is practised and those in which the animals normally live.

These experiments suggest that *Drosophila* does not follow the rules formulated by Hammond (1947) with respect to the selection of livestock on high or low planes of nutrition. Naturally direct extrapolation from the growth of an insect to that of a mammal may be unwarranted; nevertheless, certain general observations are worth noting. It is true that selection on a high plane of nutrition has so far led to the greater absolute response to selection for large body size, but the difference due to selection is not maintained when the animals are grown on a low plane. Also, differences produced by selection on a poor diet are proportionally less when the diet is improved, while adaptation to the low-plane conditions may reduce the possibility of increasing body size by further selection under better conditions. The rather crude term 'plane of nutrition' can be a source of confusion, since it rather implies that nutrition can be graded on a continuous quantitative scale. Although the gross food intake can be varied quantitatively, qualitative differences in the factors which limit growth are bound to occur on different 'planes', and thus provide a basis for the selection of gene combinations which affect metabolism in different ways.

From the characteristic differences in selection response, I have inferred that selection on different diets alters growth and metabolism in different ways. By comparing the growth of these strains on different controlled diets it should be possible to test this inference directly. The next paper in this series will take up this problem.

SUMMARY

1. Strains from a cage population of *Drosophila melanogaster* were selected for increased body size on the live yeast medium and on two aseptic synthetic media, (1) deficient in protein and (2) with all nutrients reduced to one-third the normal concentration required for growth to normal size. Both these media reduce body size by about 25%.

2. In two strains, mass selected on the live yeast medium, the response continued fairly steadily for at least sixteen generations, when the experiment was discontinued. By this time body size had been increased by some 30%.

3. On the sub-optimal diets the number of selected parents per generation was twice as great as on the live yeast medium, for technical reasons, but the response ceased abruptly after seven or eight generations of selection at a level considerably below that attained by the strains selected on the more favourable diet. Also, when selection was continued on the live yeast diet, no further progress occurred.

4. Flies selected on the different diets and also unselected flies have been grown on the alternative conditions to see how the deviation from unselected is affected. For both strains selected on sub-optimal diets, the deviation from unselected is appreciably greater on the medium used for selection than on the live yeast medium.

5. The response to selection for larger body size on deficient diets can be attributed partly to better adaptation to these conditions. This inference is supported by several lines of evidence. The within-culture variance, which is clearly greater when selected flies are grown on deficient diets, declines with effective selection. Also in the low-protein strain, for which data are available, the duration of the larval period is shortened in the early stages of selection, while egg production considerably exceeds that of unselected flies grown on the same diet.

6. Other effects, of the kind normally selected for on the live yeast medium, also contribute to the variation and selection response on the deficient diets. At first they appear to act more or less independently of the genetic changes which favour increased size via improved adaptation to the diet, but continued selection soon leads to mutual incompatibility between the alternative pathways in growth. Since no further progress occurred when selection was continued on the live yeast medium, the earlier selection had probably lowered the level of adaptation to the live yeast medium. A new genetic situation had been created in which it was impossible to gauge the amount of further progress by reference to the behaviour of strains selected on the live yeast medium from the beginning.

7. Estimates of heritability, based on cumulated selection differentials, are rather similar in the different diets and range between 0·30 and 0·38. On the live yeast medium, the estimate provides a fair guide to future progress, whereas, on the deficient diets, the predictive value is nil since response ceases immediately after the generations which provide the data for the estimates.

8. By comparing the deviation from unselected on the media used for selection and also the other media, alternative estimates of genetic correlation in performance in different conditions can be computed. The estimates were sufficiently divergent to cast doubt on the practical utility of the statistical procedure, which takes no account of the likelihood that individual variation in body size in different environment represents to greater or lesser degree the effects of segregation on different processes of growth and metabolism.

9. Since the course of selection is influenced by nutritional conditions, comparisons of response to selection for the same 'character' such as body size, in populations or species adapted to different conditions, must allow for the likelihood that unequal differences between the conditions in which selection is carried out and those in which the animal normally lives may be an important cause of differences in response.

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Genetics of resistance to *Amphorophora rubi* (Kalt.) in the raspberry

II. THE GENES A_2–A_7 FROM THE AMERICAN VARIETY, CHIEF

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1. INTRODUCTION

In Britain the rubus aphid, *Amphorophora rubi* (Kalt.), is the vector of the raspberry diseases mosaic 1 (veinbanding), mosaic 2, leaf mottle, leaf spot, and yellow blotch (Cadman, 1951, 1952*a*, 1952*b*, 1954; Cadman & Harris, 1952). On the American continent this aphid has been shown to transmit black raspberry necrosis, leaf mottle, yellow mosaic, and rubus yellow net (Stace-Smith, 1954, 1955*a*, 1955*b*). In addition, a limited number of raspberry viruses are carried by *Aphis idaei* v.d.G. Clearly, effective resistance to aphids could be of considerable value in the raspberry.

In Part I of this series the resistance of the raspberry variety, Baumforth A, to *A. rubi* strains 1 and 3 was shown to be controlled by a single dominant gene A_1 linked with the normal allele of a semi-lethal gene, *fr*, the crossover value being approximately 3·3% (Knight, Keep & Briggs, 1959).

2. STRAINS OF *AMPHOROPHORA RUBI* ON EUROPEAN RASPBERRIES

In the course of the work reported in Part I of this series and in the present paper, the existence on European raspberries of several distinct strains of *A. rubi* was recognized (Briggs, 1959). These are listed below for convenience of reference.

European raspberry strain 1 is delineated by Briggs by reason of its inability to breed on plants carrying A_1 or on 87/6, a seedling of Chief. In relation to the work reported in this paper the strain can now be more closely defined as being unable to breed on plants carrying any of the following genes: A_1, A_5, A_6, and A_7.

European raspberry strain 2 breeds on plants carrying A_1 but not on 87/6. The resistance of 87/6 to this strain is shown in this paper to depend on the series A_2, A_3 and A_4. A_2 alone confers full resistance; A_3 and A_4 are dominant complementaries which together confer full resistance, as does the combination A_1A_3.

European raspberry strain 3 is capable of some reproduction on 87/6 but is unable to breed on plants carrying A_1. In terms of the genes described in this paper, strain 3 aphids are not affected by any of the genes A_2–A_7 individually, but when all six of these genes are present the aphids cease to thrive, although some are capable of reproduction and growth to maturity on such plants.

3. DESCRIPTION OF RASPBERRY VARIETIES USED

Chief.—According to Brooks & Olmo (1949) the American raspberry variety Chief arose from a self of Latham, although from the data given later in this paper

Chief must have been an outcross and not a self. Latham was bred from the cross King × Loudon, the latter deriving from Turner × Cuthbert, both of which, according to Hedrick (1925), probably arose from varieties being grown in England at the beginning of the nineteenth century. Hedrick's illustration of the other parent of Latham, King, shows it to have been a typical American variety. Latham, the immediate parent of Chief, was thus a hybrid between *Rubus idaeus* subsp. *strigosus*, the American red raspberry, and *R. idaeus vulgatus*, the European raspberry. It is shown later in this paper that Chief is resistant to strains 1 and 2 of *A. rubi*. This variety was shown to be susceptible to *A. rubi* in America by Schwartze & Huber (1937).

87/6.—Family 87, an open-pollinated progeny of Chief, was raised by N. H. Grubb at East Malling in 1950 from seed supplied by Dr G. L. Slate of the New York State Agricultural Experiment Station, Geneva. 87/6 is a clonally propagated single plant selection from this family.

Baumforth A L3/1.—An obsolete variety of unknown origin carrying the gene A_1 for resistance to strains 1 and 3 of *A. rubi*. Certain characteristics of this variety suggest that it derives, at least in part, from *R. idaeus strigosus*.

P1.—A basic inbred line deriving from Grubb's 30/8 (Grubb & Wood, 1954), itself an F_2 seedling of a hybrid between a self of Pyne's Royal and a self of Lloyd George. For convenience 30/8 and successive selfed selections in this breeding 'line' have been called P1. These successive selections have all proved to be susceptible to strains 1, 2, and 3 of *A. rubi*.

4. RESISTANCE TO *A. RUBI* STRAIN 1

Chief and its derivative 87/6

In 1950 Briggs and Taylor tested ninty-eight seedlings of Chief by mass inoculation with adult individuals of *A. rubi* reared from a batch collected in the field at East Malling. Chief itself was not available for testing at that time and this seed, kindly supplied by Dr G. L. Slate of Geneva, New York, was of open-pollinated origin. None of these seedlings became colonized in this test although other seedlings growing in the same frame and obtained from open-pollinated Devon showed nearly 100% infestation.

During the three years 1952 to 1954, eleven clonally propagated plants of this same family derived from Chief were tested by Briggs and Keep. One of these plants (87/6) had been more extensively tested than the others and this was selected by the present writers for further work.

In 1955, six clonally propagated plants of 87/6 were put into a replicated test against controls of P1; Baumforth A, Landmark, and other types were included. Ten adult apterous strain 1 aphids were placed on each plant and counts made 14 days later showed a total of 1,311 aphids on P1, 2 on 87/6, 4 on Landmark, and 1 on Baumforth A.

Chief was not available for testing until 1955, when a single plant of this variety was inoculated with twenty *A. rubi* apterae (strain 1) of all ages; none were present 15 days later. A further 100 aphids were put on, and again none remained after

6 days. Finally, approximately 500 apterae of all ages were used: 2 days later, 1 remained, and this was no longer present on the next examination made a week later. Plants of a number of susceptible varieties were included in this same test, and these were colonized freely.

87/6 selfed

A self-bred progeny of 87/6 was grown in 1955 and the young seedlings (Family 135) all proved resistant. A second self-bred progeny of 87/6 was grown and tested in 1957 (Family 205) and in this larger family segregation into two classes 'resistant' and 'susceptible' occurred and there were no cases in which the classification was in doubt (Table 1).

Table 1. *Classification of the selfs of 87/6*

Family no.	Observed		Expected 63:1				Expected 15:1			
	Res.	Sus.	Res.	Sus.	χ^2	*P* (approx.)	Res.	Sus.	χ^2	*P* (approx.)
135	43	0	42·33	0·67	0·68	0·45	40·31	2·69	2·87	0·10
205	117	5	120·09	1·91	5·10	0·03	114·37	7·63	0·96	0·30
Totals	160	5	162·42	2·58	5·78	0·06	154·68	10·32	3·83	0·15

The data in Table 1 give slightly better agreement with a 15 : 1 interpretation than with 63 : 1, where the total χ^2 is calculated for the two separate families. If these two families are taken together as a single sample, χ^2 figures of 2·31 for a 63 : 1 expectation and 2·92 for a 15 : 1 are obtained, corresponding with probabilities of 0·15 and 0·08 respectively. Thus, although on this basis the data are in somewhat better agreement with a hypothesis of control by three dominant genes, the possibility of digenic control is not excluded.

87/6 × P1

In 1955, reciprocal crosses of 87/6 by the susceptible variety P1 were grown and tested for resistance. There were two 'doubtful' cases in Family 137. Both of these plants were included in the 'resistant' group, since although in each case an aphid deposited nymphs on them, these nymphs remained on the plants for only a few days. Apart from these two cases the phenotypes were again clear (Table 2), and the distributions agreed closely with a three-gene interpretation. In Family 137 the observed distribution was 45 : 5, but in the course of progeny testing it was found that one 'resistant' plant had been misclassified since it gave only susceptible progeny. The figures in Table 2 were therefore altered to 44 : 6.

Table 2. *Classification of progenies of 87/6 × P1*

Family no.	Parentage	Observed		Expected 7:1		χ^2	*P* (approx.)
		Res.	Sus.	Res.	Sus.		
136	P1 × 87/6	42	8	43·75	6·25	0·56	0·5
137	87/6 × P1	44	6	43·75	6·25	0·01	0·9
Totals		86	14	87·50	12·50	0·57	0·75

The distributions in Table 2 agree much more closely with a three-gene interpretation ($P = 0{\cdot}75$) than with a two-gene hypothesis ($\chi^2 = 6{\cdot}67; P = 0{\cdot}04$). If the two families are treated as a single unit, χ^2 becomes 0·21 and P is 0·65 on the 7 : 1 basis whereas the divergence from a 3 : 1 ratio is highly significant ($\chi^2 = 6{\cdot}45; P = 0{\cdot}01$).

First backcross to P1

Ten resistant plants in Families 136 and 137 (Table 2) were crossed with the inbred parent line P1. Five of the progenies were tested in 1957 (Table 3). Four of these families gave clear 1 : 1 ratios indicating the presence of a single dominant resistance gene in each case, and the fifth gave a 3 : 1 ratio indicating the presence of two such genes.

Table 3. *Classification of first backcross to P1*

Family no.	Parentage	Observed		Expected		χ^2	P (approx.)
		Res.	Sus.	Res.	Sus.		
Families carrying one major resistance gene (1:1)							
209	P1 × 136/9	24	23	23·5	23·5	0·02	0·9
215	137/6 × P1	24	26	25·0	25·0	0·08	0·8
217	137/35 × P1	25	20	22·5	22·5	0·56	0·5
208	P1 × 136/6	41	37	39·0	39·0	0·21	0·7
Totals		114	106	110·0	110·0	0·87	0·95
Family carrying two major resistance genes (3:1)							
206	P1 × 136/2	40	17	42·75	14·25	0·71	0·4

Five additional backcross progenies were grown but these were discarded because of contamination with a new strain of *A. rubi*. Owing to a shortage of aphids hatched from eggs in the insectary, aphids were collected from the field and used for testing at the beginning of the 1957 season. To conserve aphids, adults which had deposited five young on a seedling under test were moved to another seedling. Moreover, the aphids which had passed through all stages on such seedlings and become adult (the normal criterion of susceptibility) were then transferred to other seedlings and used for testing for resistance. These conditions imposed a strong selection pressure in favour of any race of *A. rubi* able to colonize plants carrying resistance genes from 87/6. This selection sieve resulted in the expansion of a strain of aphids which, unknown to the writers, must have existed at a low level in the field population, and, in consequence, families which on sample tests early in the season had shown promise of giving clear ratios, gradually 'dropped back' in their ratios as determined on subsequent samples (due to the expansion of the 'new' strain), until they appeared to comprise only susceptible plants. This new race of *A. rubi*, named strain 3 by Briggs (1959), thus vitiated most of the work on the first backcross progenies. By the time the contamination had been detected and eradicated, it was too late in the season to repeat the full series of tests with uncontaminated strain 1 aphids, and such tests had to be limited to the five families classified in Table 3.

Baumforth A × 87/6

Seedlings of a cross between Baumforth A and 87/6 were tested in 1956 (Table 4). Baumforth A is known to be of $A_1 a_1$ genotype, so that this hybrid population would be expected to give a 7 : 1 or 15 :1 ratio according to whether 87/6 is heterozygous for two or for three dominant resistance genes.

Table 4. *Classification of Baumforth A × 87/6 F_1*

	Observed		Expected 15:1				Expected 7:1			
Family no.	Res.	Sus.	Res.	Sus.	χ^2	*P* (approx.)	Res.	Sus.	χ^2	*P* (approx.)
163	91	9	93·75	6·25	1·29	0·25	87·50	12·50	1·12	0·3

The distributions in Table 4 lend themselves equally to interpretation on either a two-gene or a three-gene basis. It is shown in the next section that these genes are distinct from A_1.

Evidence for independence of A_1 from the strain 1 resistance genes in 87/6

The gene A_1 confers near immunity to strain 3 aphids; since, however, plants of 87/6 can be colonized by this strain, it is almost certain that 87/6 does not carry A_1. Moreover, as previously noted, first backcross progenies on test in 1957 proved entirely susceptible to strain 3. Since ten progenies were involved, the chances of one or more of them carrying A_1, had this been present in 87/6, would have been of the order of 999 : 1.

Discussion

The distribution in the self-bred progenies of 87/6 (Table 1) and in the F_1 of Baumforth A × 87/6 (Table 4) are equally open to interpretation of control of 87/6 resistance by two or by three genes. The segregation ratios of the F_1's of 87/6 × P1 (Table 2), on the other hand, strongly support the three-gene hypothesis and differ widely from expectation on a digenic basis. The five first-backcross progenies (Table 3) gave 1 : 1 and 3 : 1 ratios only. The absence of 7 : 1 ratios in these families does not preclude the three-gene hypothesis, since in a sample of only five progenies the chance of finding one segregating for all three genes is only about 50%.

In view of the 7 : 1 ratios given by 87/6 × P1 and the reciprocal cross (Table 2) a three-gene interpretation is considered valid, and these genes have been designated A_5, A_6 and A_7.

The discovery of strain 3 of *A. rubi*, against which these genes are ineffective, made it imperative for plant breeding purposes to concentrate on the gene A_1, since this gives near immunity to both strains 1 and 3. All further work on the isolation and utilization of A_5, A_6 and A_7 was therefore discontinued.

5. RESISTANCE TO *A. RUBI* STRAIN 2

In Britain, the only form of *A. rubi* so far found capable of colonizing plants carrying the gene A_1 is that designated 'strain 2' by Briggs (1959). When this strain was discovered by the writers in 1955, sources of resistance to it were at once sought

amongst the range of raspberry varieties and *Rubus* spp. carried at East Malling. Both Chief and its self-bred derivative, 87/6, proved resistant; P1 proved fully susceptible.

87/6 selfed

A self-bred progeny of 87/6 (Family 205) was tested with strain 2 aphids in 1957 and clear-cut segregation was obtained (Table 5).

Table 5. *Classification of the selfs of 87/6*

Family no.	Observed		Expected (57:7)		χ^2	P (approx.)
	Resistant	Susceptible	Resistant	Susceptible		
205	88	10	87·28	10·72	0·06	0·8

Although the distribution in Family 205 agrees closely with expectation on a 57 : 7 basis, it does not differ significantly from 15 : 1 ($\chi^2 = 2{\cdot}6$; $P = 0{\cdot}1$). A 57 : 7 ratio suggests control by three dominant genes, two of which are complementary.

87/6 × P1 and the first backcross to P1

F_1 families of 87/6 × P1 and the reciprocal were not tested with strain 2 aphids in the insectary, since these families were already planted out in the field by the time strain 2 became available in sufficient quantity for resistance testing. A rough test was accordingly made by topping young canes of each plant and testing these tops in the insectary, keeping the cut ends immersed in water. This rough test was not expected to give an accurate ratio, but merely to give an indication of possible resistant plants amongst which selections for backcrossing to P1 could be made. Five plants, thought to be resistant, were selected in this way and backcrossed to P1. The classification of their progenies is shown in Table 6.

Table 6. *Classification of first backcross*

Family no.	Parentage	Observed		Expected		χ^2	P (approx.)
		Res.	Sus.	Res.	Sus.		
				1:3			
215	137/6 × P1	14	36	12·50	37·50	0·24	0·6
216	137/33 × P1	7	18	6·25	18·75	0·12	0·7
Totals		21	54	18·75	56·25	0·36	0·85
				1:1			
217	137/35 × P1	19	26	22·50	22·50	1·08	0·3
				5:3			
206	P1 × 136/2	8	5	8·13	4·88	0·01	0·9
208	P1 × 136/6	50	29	49·38	29·63	0·01	0·9
Totals		58	34	57·51	34·51	0·02	0·99

Second backcross to P1

Three plants in Family 215 and three in Family 217 (Table 6) all resistant to strain 2 were again crossed as males with susceptible derivatives of P1 (Family 166). One of these plants (No. 217/2) gave a hybrid progeny all of which were susceptible, indicating either that the parent plant was misclassified or that a berry from the maternal parent had been picked and sown in error. The numbers in Tables 6 and 9 have accordingly been adjusted by transferring one S^1R^2 plant to the S^1S^2 phenotype (S^1R^2 denotes susceptibility to strain 1 aphids and resistance to strain 2, etc.). These second backcross families gave reasonably close approximations to the 1 : 3 and 1 : 1 ratios expected (Table 7), except for Families 402 and 404 in which there were a large number of plants whose classification was doubtful. In Family 402, the thirty-two plants in the 'resistant' group included twelve plants which had been graded as 'doubtfully resistant' and in several of the others the resistance was far from sharply defined; this difficulty of classification was undoubtedly due to the plants having become somewhat pot-bound whilst awaiting their time for testing. Similarly, the forty 'resistant' plants in Family 404 included eight plants regarded as 'doubtfully resistant'. That some plants became pot-bound was due to the need to make maximum use of the available bench space in order to get through a crowded programme. Hence 'potting on' into larger pots was often of necessity delayed, especially where tests with more than one strain of aphid were required.

In addition to the progenies involving Families 215 and 217, eight plants of Family 208 were used as males in backcrossing to P1. The response of these eight plants to strain 2 was uncertain, owing to an error in labelling in the field, and two of the progenies proved to be entirely susceptible. Three progenies gave 1 : 3 ratios and three gave 1 : 1 ratios (Table 7).

Table 7. *Classification of second backcross*

Family no.	Parentage	Observed		Expected		χ^2	P (approx.)
		Res.	Sus.	Res.	Sus.		
				1:3			
402	166/9 × 215/3	32	68	25·00	75·00	2·61	0·1
403	166/10 × 215/6*	3	14	4·25	12·75	0·49	0·5
404	166/90 × 215/5	40	57	24·25	72·75	13·64	0·001
398	P1 × 208/9	13	52	16·25	48·75	0·87	0·4
399	P1 × 208/10	7	28	8·75	26·25	0·46	0·5
400	P1 × 208/11	8	11	4·75	14·25	2·96	0·1
Totals		103	230	83·25	249·75	21·03†	0·001†
				1:1			
406	166/9 × 217/4	33	45	39·00	39·00	1·85	0·2
407	166/10 × 217/7	44	38	41·00	41·00	0·44	0·5
393	P1 × 208/3	18	17	17·50	17·50	0·03	0·85
395	P1 × 208/2	20	15	17·50	17·50	0·71	0·4
396	P1 × 208/7	11	19	15·00	15·00	2·13	0·15
Totals		126	134	130·00	130·00	5·16	0·4

* Plant No. 215/6 subsequently proved to be triploid.

† Omitting Family 404 the total χ^2 is 7·39 and $P = 0·2$ approx.

Self-bred progenies from the first backcross

Self-bred progenies of first backcross plants Nos. 215/3, 215/8, 217/4 and 217/7 (Table 6) gave reasonable approximations to expectation on a 9 : 7 and 3 : 1 basis (Table 8) confirming the 1 : 3 and 1 : 1 ratios obtained in Table 7. Eleven plants of Family 208 were selfed also. The response of these plants to strain 2 aphids was uncertain (as noted earlier) and the progenies from three of them were all susceptible; of the remaining eight plants, three gave 9 : 7 ratios and five gave 3 : 1 ratios (Table 8).

Table 8. *Classification of F_2 of first backcross*

Family no.	Parentage	Observed		Expected		χ^2	P (approx.)
		Res.	Sus.	Res.	Sus.		
				9:7			
388	215/3 Self	20	13	18·56	14·44	0·26	0·6
389	215/8 Self	14	13	15·19	11·81	0·21	0·6
382	208/9 Self	32	22	30·38	23·63	0·19	0·7
383	208/10 Self	24	14	21·38	16·63	0·73	0·4
384	208/11 Self	30	25	30·94	24·06	0·07	0·8
Totals		120	87	116·45	90·57	1·46	0·9
				3:1			
391	217/4 Self	9	8	12·75	4·25	4·41	0·04
392	217/7 Self	45	18	47·25	15·75	0·42	0·5
376	208/2 Self	24	8	24·00	8·00	—	—
377	208/3 Self	45	12	42·75	14·25	0·47	0·5
378	208/4 Self	28	7	26·25	8·75	0·47	0·5
379	208/5 Self	16	5	15·75	5·25	0·02	0·9
381	208/7 Self	38	17	41·25	13·75	1·02	0·3
Totals		205	75	210·00	70·00	6·81	0·5

Discussion

The 57 : 7 ratio obtained in the self-bred progeny of 87/6 suggests that control of resistance to strain 2 aphids in this variety depends on three genes, one being a strong dominant capable by itself of conferring full resistance and the other two being dominant complementaries. The backcross ratios (Tables 6 and 7) of 1 : 3, 1 : 1 and 5 : 3 support this interpretation, which is further confirmed by the 9 : 7 and 3 : 1 ratios obtained in F_2 progenies of the first backcross (Table 8).

These genes are shown in the next section to be distinct from A_5, A_6 and A_7, and they have been called A_2, A_3 and A_4 respectively, A_2 by itself conferring full resistance and A_3 and A_4 being dominant complementaries.

6. TESTS OF INDEPENDENCE OF THE STRAIN 1 RESISTANCE GENES A_5–A_7 FROM THE STRAIN 2 SERIES A_2–A_4

In testing for independence of the strain 1 resistance genes from those affecting strain 2, many of the insectary tests in 1957 were invalidated because of contamination of the strain 1 aphid stock with strain 3 aphids. This point has already been

discussed; it threw much of the work out of phase so that it was not always possible to test the *same* plants in each family with both strains 1 and 2. Data from the families in which the same plants were tested with both strains, are given in Table 9.

Table 9. *Classification of selfs and backcrosses for resistance to* A. rubi *strains 1 and 2*

	Family numbers										
	205			208			215			217	
* Phenotypes	Observed	Expected (3591:441:57:7)	Expected with linkage	Observed	Expected (5:3:5:3)	Expected with linkage	Observed	Expected (1:3:1:3)	Expected with linkage	Observed	Expected (1:1:1:1)
R^1R^2	87	85·92	86·08	33	24·69	28·63	12	6·25	11·25	13	11·25
R^1S^2	9	10·55	10·39	7	14·81	10·86	12	18·75	13·75	12	11·25
S^1R^2	1	1·36	1·20	18	24·69	20·74	2	6·25	1·25	6	11·25
S^1S^2	1	0·17	0·33	21	14·81	18·76	24	18·75	23·75	14	11·25
Totals	98	98·00	98·00	79	79·00	78·99	50	50·00	50·00	45	45·00
χ^2		4·39	1·58		11·32	2·68		12·08	0·72		3·44
P (approx.)		0·2	0·7		0·01	0·5		0·01	0·85		0·3

* Superscripts denote strains of *A. rubi* to which the plants are resistant (R) or susceptible (S).

Family 205 = 87/6 selfed ($A_2a_2A_3a_3A_4a_4A_5a_5A_6a_6A_7a_7$) (Tables 1 and 5).
208 = P1 × 136/6 (sus. × $A_2a_2A_3a_3A_4a_4A_5a_5$) (Tables 3 and 6).
215 = 137/6 × P1 ($A_3a_3A_4a_4A_5a_5$ × sus.) (Tables 3 and 6).
217 = 137/35 × P1 (A_2a_2 + a het. gene of A_5–A_7 group × sus.) (Tables 3 and 6).

The linkage ratios are calculated assuming 10% recombination between one of the complementary genes, A_3A_4, and A_5.

Family 205 (the self-bred progeny of 87/6) was shown to segregate for genes A_5, A_6 and A_7 (Table 1) and also for A_2, A_3 and A_4 (Table 5). The figures in Table 9 show reasonably good agreement with expectation on the basis that there is no interaction (apart from linkage) between these strain 1 and strain 2 resistance genes.

The first backcross families 208, 215 and 217 all gave 1 : 1 ratios when inoculated with strain 1 aphids (Table 3); they gave 5 : 3, 1 : 3 and 1 : 1 ratios respectively when tested with strain 2 (Table 6). From Table 9 it is clear that Family 217 agrees well with expectation on the basis that it carried the dominant gene A_2 together with a single gene of the A_5–A_7 group.

Families 208 and 215 agree less well with expectation, Family 215 which carries the complementaries A_3 and A_4 unaccompanied by A_2 (Table 6) being particularly skew. Evidently one of the genes of the A_5–A_7 group is linked with either A_3 or A_4, the distributions in Family 215 suggesting a crossover value of about 10%. For convenience this linked gene is considered to be A_5.

7. TESTS OF INDEPENDENCE OF A_1 FROM THE A_2–A_4 SERIES

In 1956, a family derived from crossing Baumforth A with 87/6 was tested with strains 1 and 2 of *A. rubi* (Table 10). The variety Baumforth A is known to be of A_1a_1 genotype (Knight, Keep & Briggs, 1959) and hence to be resistant to strains 1 and 3 but susceptible to strain 2.

Table 10. *Classification of Family 163, Baumforth A × 87/6 F_1*

Phenotypes	Observed	Expected 83:37:5:3	Expected with linkage
R^1R^2	73	64·84	65·47
R^1S^2	18	28·91	28·28
S^1R^2	6	3·91	3·28
S^1S^2	3	2·34	2·97
Totals	100	100·00	100·00
χ^2		6·45	6·86
P		0·09	0·08

The 'expected' figures in Table 10 are based on the assumption that the genotypes A_2, A_3A_4 and A_1A_3 are resistant to strain 2 aphids. The 'expected' figures for linkage are calculated on the basis of a 10% c.o.v. for A_3 or A_4 with A_5.

Support for the assumption that A_1 interacts with one of the two complementaries, A_3A_4, is given by the results from Family 279, which was tested in 1958. This family was obtained by crossing 136/6, known to be heterozygous for $A_2A_3A_4$ (Table 6), with 130/42, a homozygous A_1A_1 plant (Table 11).

Table 11. *Strain 2 tests on* $A_2a_2A_3a_3A_4a_4 \times A_1A_1$

	Observed		Expected (3:1)			
Family	Res.	Sus.	Res.	Sus.	χ^2	P (approx.)
279	56	15	53·25	17·75	0·57	0·45

Had there been no interaction between A_1 and one of the two complementaries A_3A_4 this family would have given a 5 : 3 ratio of resistant to susceptible plants, when tested with strain 2 aphids. The interaction of A_1 with one of these genes has converted this ratio to 3 : 1 and the distribution of 56 : 15 obtained differs significantly from expectation on a 5 : 3 basis ($\chi^2 = 8{\cdot}11$; $P = 0{\cdot}007$). There is no means of distinguishing the action of A_3 from that of A_4 other than definition in terms of this interaction with A_1. For convenience of definition, A_3 is regarded as this interacting gene.

From the existing data it is impossible to determine whether A_5 is linked with A_3 or with A_4. It is only possible to distinguish A_3 from A_4 in the presence of A_1, and since A_1 confers resistance against both strain 1 and strain 3, the presence or absence of A_5 is then not detectable. This question could be determined by crossing $A_1a_1A_3a_3$ plants with A_5a_5. This would give 2 $R^1R^2R^3$: 2 $R^1S^2R^3$: 2 $R^1S^2S^3$: 2 $S^1S^2S^3$ (using superscripts of R and S to denote resistance or susceptibility to strains 1, 2, and 3). By crossing a number of these $R^1S^2S^3$ plants with A_1A_1 and

testing with strain 2 aphids, it would be possible to detect whether or not there was an excess of A_3A_5 plants amongst them, since A_1A_3 is resistant to strain 2 whereas A_1a_3 plants are susceptible. Such a method of proving which of the two genes is linked with A_5 would, however, involve an unjustifiable amount of work in view of the fact that *A. rubi* strain 3 has superseded strain 1 for plant breeding purposes.

8. GENERAL DISCUSSION

Origin of the resistance genes found in Chief

Slate (1935) and Brooks & Olmo (1949) state that Chief was selected from the self-bred progeny of the American variety Latham. In 1958 a self-bred progeny of Latham was grown; 20 of these plants were tested with strain 1 aphids and 2 showed weak resistance while 18 were more or less fully susceptible. A further 20 plants tested with strain 2 aphids, showed 1 resistant, 2 weakly resistant and 17 fully susceptible. From this it seems unlikely that Latham carries any major genes for resistance to either of these strains of *A. rubi*.

Since six dominant resistance genes have been located in Chief it follows that this variety cannot be a self-bred seedling of Latham but that it must have been an outcross, an explanation much more in keeping with the fact that Chief proved to be heterozygous for all six of these genes.

Economic significance of the individual resistance genes

The discovery, in 1957, of *A. rubi* strain 3 showed the genes A_5, A_6 and A_7 to be of little commercial importance. Since A_1 confers strong resistance to strain 1 and strain 3 aphids, this gene was chosen as the main basis of raspberry breeding designed to achieve field immunity.

Strain 2 aphids can colonize plants carrying A_1 with or without A_5, A_6 and A_7. However, adequate resistance to this aphid strain can be achieved by using A_2 alone, A_3 combined with A_4, or A_1 combined with A_3 (Table 12).

Table 12. *Interaction of resistance genes and aphid strains*

	Response to *A. rubi*		
Raspberry genes	Strain 1	Strain 2	Strain 3
A_1	R	S	R
A_2	S	R	S
A_3+A_4	S	R	S
A_5	R	S	S
A_6	R	S	S
A_7	R	S	S
A_1+A_2	R	R	R
A_1+A_3	R	R	R
A_1+A_4	R	S	R

For plant breeding purposes, the simplest control of these three strains of *A. rubi* will be achieved by using the combination A_1A_2 or A_1A_3. A programme involving A_1 and A_2 would require testing of progenies with both strain 2 and strain 3 aphids; the use of the combination A_1A_3 would require tests with strain 2 aphids only.

Both the combinations $A_1 A_2$ and $A_1 A_3$ will be used until field tests are available on a sufficient scale to show whether there is any difference in resistance.

Minor-gene resistance

The method of breeding raspberries resistant to *A. rubi* has been based on the use of major resistance genes from Baumforth A and Chief. Nevertheless, in the course of the search for genes of suitably large effect, a number of instances were found of resistance apparently controlled by minor genes. The system of testing for resistance was designed for classifying seedlings as either more or less immune or fully susceptible, but intermediate levels of resistance are shown by the general behaviour of the aphids. Adults are more restless and tend to leave partially resistant seedlings after depositing only a few nymphs. These nymphs may linger for variable periods on such plants, often feeding on the stem and lower leaves rather than on young leaves. Occasionally they grow to maturity, but the unsuitability of the host plant is usually evident from the smallness of the resulting adult.

Tests with strain 1 aphids on self-bred progenies of Reid's AR1 showed a continuous range, as indicated by aphid behaviour, from a few plants with full resistance to others showing full susceptibility. Some derivatives evinced a measure of resistance to strain 2 and strain 3 aphids, but there was no correlation between resistance to these two aphid strains. Moreover, field counts on a self-bred progeny of Reid's AR1 showed no significant differences between populations on plants previously classified in the insectary as 'resistant', 'intermediate' or 'susceptible' to strain 1 aphids.

Numerous field counts on Norfolk Giant have shown it to be partially resistant. Selfs of Norfolk Giant, tested in the insectary with strain 1 aphids, gave a response similar to that of the Reid's AR1 progeny, both in the insectary and in the field. Similarly, self-bred progenies deriving from Baumforth B showed a continuous range from resistance to susceptibility when tested in the insectary with strain 3 aphids, suggesting that this variety, also, carries minor resistance genes.

Relative value of major- and minor-gene resistance

In breeding for resistance to pests and diseases it is often suggested that plant breeders should use resistance controlled by minor-gene complexes rather than oligogenic resistance, on the ground that the more complex the resistance, the less is it likely to succumb to new biologic strains of the pest or pathogen. This, though true of certain pests and diseases, is by no means universally applicable. Thus the high resistance of the raspberry variety Lloyd George under field conditions in North America has been shown by Schwartze & Huber (1939) to be simply inherited and this resistance has been maintained there for about 30 years without breaking down.

In the raspberry, resistance to *A. rubi* can be controlled by major genes or by minor ones, but our evidence to date suggests that minor genes confer *resistance*, rather than immunity, and mere resistance, unless it approached immunity, would be valueless in preventing virus infection and spread in a crop. Moreover, minor-gene resistance to one strain of *A. rubi* does not necessarily confer resistance to another.

There can be little doubt that resistance to *A. rubi* belongs to the group of resistances best achieved by the use of major genes. This is fortunate because few raspberries would tolerate the repeated selfing required in integrating minor-gene complexes, and the difficulties involved in handling such complexes over and above those controlling yield and fruit quality would be considerable. Moreover, the method of testing would have to be more sensitive, thus inevitably restricting the number of plants that could be handled.

SUMMARY

The American raspberry variety Chief is shown to carry three dominant genes, A_5, A_6 and A_7, each capable of conferring strong resistance to *Amphorophora rubi* strain 1.

Chief also carries three genes, A_2, A_3 and A_4, for resistance to *A. rubi* strain 2. A_2 is a dominant gene conferring full resistance by itself; A_3 and A_4 are dominant complementaries, neither gene by itself having any effect on resistance. A_5 is linked with either A_3 or A_4 with a crossover value of 10%.

The gene A_1 from Baumforth A, which confers resistance to strains 1 and 3, when combined with A_3 gives resistance to strain 2 also. Thus the three strains of *A. rubi* at present recognized on raspberries in Britain can be controlled by using either the combination $A_1 A_2$ or $A_1 A_3$.

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Discussion on the mutagenic effects of alkylating agents

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Recently, a publication under this title has been distributed by Drs O. G. and M. J. Fahmy. On top of every page, it carries my name and not that of the authors. This has already resulted in its summary having been printed mistakenly as 'author's summary' of a paper of mine in *Biological Abstracts*. Since this curious publication is likely to lead to more confusion, its origin is explained below.

In 1957, the New York Academy of Sciences held a symposium on 'Biological Effects of Alkylating Agents', at which I had been asked to read a paper on the mutagenic effects of these substances. Dr O. G. Fahmy, as one of the official discussants, was given five minutes in which to make a few remarks. To these, Dr I. I. Oster, the other discussant, and myself answered equally briefly. The remarks by Dr Oster and myself have been printed in the New York Academy's Symposium volume, but they now follow a paper twice the length of mine by Drs Fahmy, which the editors, without my knowledge, had substituted for the brief discussion remarks which the Drs Fahmy actually delivered. It is this paper which has now been issued as a separate reprint.

Actually, this paper had been read, but not at the Academy meeting. It formed the basis for a discussion at the Sloane-Kettering Institute, arranged at my urgent wish so as to give the Fahmys an opportunity to explain and defend their far-reaching claims. Professor Haddow, F.R.S., was in the chair, and the discussion was mainly restricted to the Drs Fahmy, Dr Oster and myself. It lasted four hours and was very lively and unrestrained. Subsequently, Professor Haddow asked me to draw up a report of this discussion for inclusion in the Symposium volume; but to publish the proceedings of a semi-private meeting at which the Fahmys had been severely criticized did not seem fair, and I refused Professor Haddow's request. I would certainly not have done so, had I been told that this would lead to the Fahmys' paper being published without the criticism to which it had been subjected.

A brief report of the meeting, drawn up by Dr Oster and myself and seen and approved by Professor Haddow and the Drs Fahmy, has been circulated privately. Copies are still available.

It seems that the Drs Fahmy have profited from this report to the extent that the printed version of their talk omits some of the most severely criticized statements and contains some additional data that do not seem to have been available at the meeting. These slight changes do not, however, affect the main conclusions which read as follows:

'Drs Fahmy have reported many radically new effects of chemicals. Geneticists who work on *Drosophila* must admire the tremendous amount of work, carried out at unprecedented speed, which was necessary for the accumulation of the data on which these claims are based. Unfortunately, the published data represent only—according to Drs Fahmy—a very small proportion of those actually obtained. In fact, hardly any of these claims can be substantiated from the published data.'